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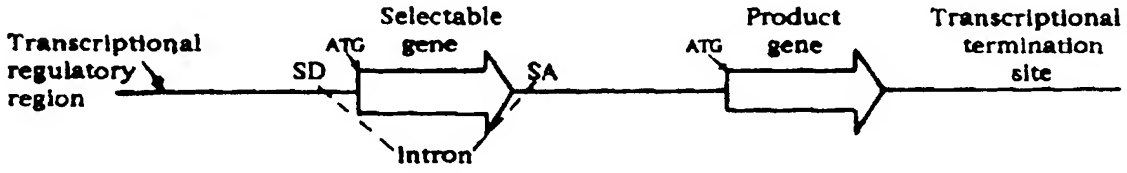

(57) Abstract

A method for selecting recombinant host cells expressing high levels of a desired protein is described. This method utilizes eukaryotic host cells harboring a DNA construct comprising a selectable gene (preferably an amplifiable gene) and a product gene provided 3' to the selectable gene. The selectable gene is positioned within an intron defined by a splice donor site and a splice acceptor site and the selectable gene and product gene are under the transcriptional control of a single transcriptional regulatory region. The splice donor site is generally an efficient splice donor site and thereby regulates expression of the product gene using the transcriptional regulatory region. The transfected cells are cultured so as to express the gene encoding the product in a selective medium comprising an amplifying agent for sufficient time to allow amplification to occur, whereupon either the desired product is recovered or cells having multiple copies of the product gene are identified.

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METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to a method of selecting for high-expressing host cells, a method of producing a protein of interest in high yields and a method of producing eukaryotic cells having multiple copies of a sequence encoding a protein of interest.

Description of Background and Related Art

The discovery of methods for introducing DNA into living host cells in a functional form has provided the key to understanding many fundamental biological processes, and has made possible the production of important proteins and other molecules in commercially useful quantities.

Despite the general success of such gene transfer methods, several common problems exist that may limit the efficiency with which a gene encoding a desired protein can be introduced into and expressed in a host cell. One problem is knowing when the gene has been successfully transferred into recipient cells. A second problem is distinguishing between those cells that contain the gene and those that have survived the transfer procedures but do not contain the gene. A third problem is identifying and isolating those cells that contain the gene and that are expressing high levels of the protein encoded by the gene.

In general, the known methods for introducing genes into eukaryotic cells tend to be highly inefficient. Of the cells in a given culture, only a small proportion take up and express exogenously added DNA, and an even smaller proportion stably maintain that DNA.

Identification of those cells that have incorporated a product gene encoding a desired protein typically is achieved by introducing into the same cells another gene, commonly referred to as a selectable gene, that encodes a selectable marker. A selectable marker is a protein that is necessary for the growth or survival of a host cell under the particular culture conditions chosen, such as an enzyme that confers resistance to an antibiotic or other drug, or an enzyme that compensates for a metabolic or catabolic defect in the host cell. For example, selectable genes commonly used with eukaryotic cells include the genes for aminoglycoside phosphotransferase (APH), hygromycin phosphotransferase (hyg), dihydrofolate reductase (DHFR), thymidine kinase (tk), neomycin, puromycin, glutamine synthetase, and asparagine synthetase.

The method of identifying a host cell that has incorporated one gene on the basis of expression by the host cell of a second incorporated gene encoding a selectable marker is referred to as cotransfectation (or cotransfection). In that method, a gene encoding a desired polypeptide and a selection gene typically are introduced into the host cell simultaneously, although they may be introduced sequentially. In the case of simultaneous cotransfectation, the gene encoding the desired polypeptide

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and the selectable gene may be present on a single DNA molecule or on separate DNA molecules prior to being introduced into the host cells. Wigler *et al.*, Cell, **16**:777 (1979). Cells that have incorporated the gene encoding the desired polypeptide then are identified or isolated by culturing the cells under conditions that preferentially allow for the growth or survival of those cells that synthesize the selectable marker encoded by the selectable gene.

The level of expression of a gene introduced into a eukaryotic host cell depends on multiple factors, including gene copy number, efficiency of transcription, messenger RNA (mRNA) processing, stability, and translation efficiency. Accordingly, high level expression of a desired polypeptide typically will involve optimizing one or more of those factors.

For example, the level of protein production may be increased by covalently joining the coding sequence of the gene to a "strong" promoter or enhancer that will give high levels of transcription. Promoters and enhancers are nucleotide sequences that interact specifically with proteins in a host cell that are involved in transcription. Kriegler, Meth. Enzymol., **185**:512 (1990); Maniatis *et al.*, Science, **236**:1237 (1987). Promoters are located upstream of the coding sequence of a gene and facilitate transcription of the gene by RNA polymerase. Among the eukaryotic promoters that have been identified as strong promoters for high-level expression are the SV40 early promoter, adenovirus major late promoter, mouse metallothionein-I promoter, Rous sarcoma virus long terminal repeat, and human cytomegalovirus immediate early promoter (CMV).

Enhancers stimulate transcription from a linked promoter. Unlike promoters, enhancers are active when placed downstream from the transcription initiation site or at considerable distances from the promoter, although in practice enhancers may overlap physically and functionally with promoters. For example, all of the strong promoters listed above also contain strong enhancers. Bendig, Genetic Engineering, 7:91 (Academic Press, 1988).

The level of protein production also may be increased by increasing the gene copy number in the host cell. One method for obtaining high gene copy number is to directly introduce into the host cell multiple copies of the gene, for example, by using a large molar excess of the product gene relative to the selectable gene during cotransfectation. Kaufman, Meth. Enzymol., **185**:537 (1990). With this method, however, only a small proportion of the cotransfected cells will contain the product gene at high copy number. Furthermore, because no generally applicable, convenient method exists for distinguishing such cells from the majority of cells that contain fewer copies of the product gene, laborious and time-consuming screening methods typically are required to identify the desired high-copy number transfectants.

Another method for obtaining high gene copy number involves cloning the gene in a vector that is capable of replicating autonomously in the host cell. Examples of such vectors include mammalian expression vectors

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derived from Epstein-Barr virus or bovine papilloma virus, and yeast 2-micron plasmid vectors. Stephens & Hentschel, Biochem. J., 248:1 (1987); Yates *et al.*, Nature, 313:812 (1985); Beggs, Genetic Engineering, 2:175 (Academic Press, 1981).

Yet another method for obtaining high gene copy number involves gene amplification in the host cell. Gene amplification occurs naturally in eukaryotic cells at a relatively low frequency. Schimke, J. Biol. Chem., 263:5989 (1988). However, gene amplification also may be induced, or at least selected for, by exposing host cells to appropriate selective pressure. For example, in many cases it is possible to introduce a product gene together with an amplifiable gene into a host cell and subsequently select for amplification of the marker gene by exposing the cotransfected cells to sequentially increasing concentrations of a selective agent. Typically the product gene will be coamplified with the marker gene under such conditions.

The most widely used amplifiable gene for that purpose is a DHFR gene, which encodes a dihydrofolate reductase enzyme. The selection agent used in conjunction with a DHFR gene is methotrexate (Mtx). A host cell is cotransfected with a product gene encoding a desired protein and a DHFR gene, and transfectants are identified by first culturing the cells in culture medium that contains Mtx. A suitable host cell when a wild-type DHFR gene is used is the Chinese Hamster Ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub & Chasin, Proc. Nat. Acad. Sci. USA, 77:4216 (1980). The transfected cells then are exposed to successively higher amounts of Mtx. This leads to the synthesis of multiple copies of the DHFR gene, and concomitantly, multiple copies of the product gene. Schimke, J. Biol. Chem., 263:5989 (1988); Axel *et al.*, U.S. Patent No. 4,399,216; Axel *et al.*, U.S. Patent No. 4,634,665. Other references directed to co-transfection of a gene together with a genetic marker that allows for selection and subsequent amplification include Kaufman in Genetic Engineering, ed. J. Setlow (Plenum Press, New York), Vol. 9 (1987); Kaufman and Sharp, J. Mol. Biol., 159:601 (1982); Ringold *et al.*, J. Mol. Appl. Genet., 1:165-175 (1981); Kaufman *et al.*, Mol. Cell Biol., 5:1750-1759 (1985); Kaetzel and Nilson, J. Biol. Chem., 263:6244-6251 (1988); Hung *et al.*, Proc. Natl. Acad. Sci. USA, 83:261-264 (1986); Kaufman *et al.*, EMBO J., 6:87-93 (1987); Johnston and Kucey, Science, 242:1551-1554 (1988); Urlaub *et al.*, Cell, 33:405-412 (1983).

To extend the DHFR amplification method to other cell types, a mutant DHFR gene that encodes a protein with reduced sensitivity to methotrexate may be used in conjunction with host cells that contain normal numbers of an endogenous wild-type DHFR gene. Simonsen and Levinson, Proc. Natl. Acad. Sci. USA, 80:2495 (1983); Wigler *et al.*, Proc. Natl. Acad. Sci. USA, 77:3567-3570 (1980); Haber and Schimke, Somatic Cell Genetics, 8:499-508 (1982).

Alternatively, host cells may be co-transfected with the product gene, a DHFR gene, and a dominant selectable gene, such as a neo^r gene. Kim

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and Wold, Cell, 42:129 (1985); Capon *et al.*, U.S. Pat. No. 4,965,199. Transfectants are identified by first culturing the cells in culture medium containing neomycin (or the related drug G418), and the transfectants so identified then are selected for amplification of the DHFR gene and the product gene by exposure to successively increasing amounts of Mtx.

As will be appreciated from this discussion, the selection of recombinant host cells that express high levels of a desired protein generally is a multi-step process. In the first step, initial transfectants are selected that have incorporated the product gene and the selectable gene. In subsequent steps, the initial transfectants are subject to further selection for high-level expression of the selectable gene and then random screening for high-level expression of the product gene. To identify cells expressing high levels of the desired protein, typically one must screen large numbers of transfectants. The majority of transfectants produce less than maximal levels of the desired protein. Further, Mtx resistance in DHFR transformants is at least partially conferred by varying degrees of gene amplification. Schimke, Cell, 37:705-713 (1984). The inadequacies of co-expression of the non-selected gene have been reported by Wold *et al.*, Proc. Natl. Acad. Sci. USA, 76:5684-5688 (1979). Instability of the amplified DNA is reported by Kaufman and Schimke, Mol. Cell Biol., 1:1069-1076 (1981); Haber and Schimke, Cell, 26:355-362 (1981); and Fedespiel *et al.*, J. Biol. Chem., 259:9127-9140 (1984).

Several methods have been described for directly selecting such recombinant host cells in a single step. One strategy involves co-transfecting host cells with a product gene and a DHFR gene, and selecting those cells that express high levels of DHFR by directly culturing in medium containing a high concentration of Mtx. Many of the cells selected in that manner also express the co-transfected product gene at high levels. Page and Sydenham, Bio/Technology, 9:64 (1991). This method for single-step selection suffers from certain drawbacks that limit its usefulness. High-expressing cells obtained by direct culturing in medium containing a high level of a selection agent may have poor growth and stability characteristics, thus limiting their usefulness for long-term production processes. Page and Snyderman, Bio/Technology, 9:64 (1991). Single-step selection for high-level resistance to Mtx may produce cells with an altered, Mtx-resistant DHFR enzyme, or cells that have altered Mtx transport properties, rather than cells containing amplified genes. Haber *et al.*, J. Biol. Chem., 256:9501 (1981); Assaraf and Schimke, Proc. Natl. Acad. Sci. USA, 84:7154 (1987).

Another method involves the use of polycistronic mRNA expression vectors containing a product gene at the 5′ end of the transcribed region and a selectable gene at the 3′ end. Because translation of the selectable gene at the 3′ end of the polycistronic mRNA is inefficient, such vectors exhibit preferential translation of the product gene and require high levels of polycistronic mRNA to survive selection. Kaufman, Meth.

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Enzymol., 185:487 (1990); Kaufman, Meth. Enzymol., 185:537 (1990); Kaufman *et al.*, EMBO J., 6:187 (1987). Accordingly, cells expressing high levels of the desired protein product may be obtained in a single step by culturing the initial transfectants in medium containing a selection agent appropriate for use with the particular selectable gene. However, the utility of these vectors is variable because of the unpredictable influence of the upstream product reading frame on selectable marker translation and because the upstream reading frame sometimes becomes deleted during methotrexate amplification (Kaufman *et al.*, J. Mol. Biol., 159:601-621 [1982]; Levinson, Methods in Enzymology, San Diego: Academic Press, Inc. [1990]). Later vectors incorporated an internal translation initiation site derived from members of the picornavirus family which is positioned between the product gene and the selectable gene (Pelletier *et al.*, Nature, 334:320 [1988]; Jang *et al.*, J. Virol., 63:1651 [1989]).

A third method for single-step selection involves use of a DNA construct with a selectable gene containing an intron within which is located a gene encoding the protein of interest. See U.S. Patent No. 5,043,270 and Abrams *et al.*, J. Biol. Chem., **264(24)**: 14016-14021 (1989). In yet another single-step selection method, host cells are co-transfected with an intron-modified selectable gene and a gene encoding the protein of interest. See WO 92/17566, published October 15, 1992. The intron-modified gene is prepared by inserting into the transcribed region of a selectable gene an intron of such length that the intron is correctly spliced from the corresponding mRNA precursor at low efficiency, so that the amount of selectable marker produced from the intron-modified selectable gene is substantially less than that produced from the starting selectable gene. These vectors help to insure the integrity of the integrated DNA construct, but transcriptional linkage is not achieved as selectable gene and the protein gene are driven by separate promoters.

Other mammalian expression vectors that have single transcription units have been described. Retroviral vectors have been constructed (Cepko *et al.*, Cell, 37:1053-1062 [1984]) in which a cDNA is inserted between the endogenous Moloney murine leukemia virus (M-MuLV) splice donor and splice acceptor sites which are followed by a neomycin resistance gene. This vector has been used to express a variety of gene products following retroviral infection of several cell types.

With the above drawbacks in mind, it is one object of the present invention to increase the level of homogeneity with regard to expression levels of stable clones transfected with a product gene of interest, by expressing a selectable marker (DHFR) and the protein of interest from a single promoter.

It is another object to provide a method for selecting stable, recombinant host cells that express high levels of a desired protein product, which method is rapid and convenient to perform, and reduces the numbers of transfected cells which need to be screened. Furthermore, it is

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an object to allow high levels of single and two unit polypeptides to be rapidly generated from clones or pools of stable host cell transfectants.

It is an additional object to provide expression vectors which bias for active integration events (*i.e.* have an increased tendency to generate transformants wherein the DNA construct is inserted into a region of the genome of the host cell which results in high level expression of the product gene) and can accommodate a variety of product genes without the need for modification.

SUMMARY OF THE INVENTION

Accordingly, the present invention is directed to a DNA construct (DNA molecule) alternative terminology comprising a 5' transcriptional initiation site and a 3' transcriptional termination site, a selectable gene (preferably an amplifiable gene) and a product gene provided 3' to the selectable gene, a transcriptional regulatory region regulating transcription of both the selectable gene and the product gene, the selectable gene positioned within an intron defined by a splice donor site and a splice acceptor site. The splice donor site preferably comprises an effective splice donor sequence as herein defined and thereby regulates expression of the product gene using the transcriptional regulatory region.

In another embodiment, the invention provides a method for producing a product of interest comprising culturing a eukaryotic cell which has been transfected with the DNA construct described above, so as to express the product gene and recovering the product.

In a further embodiment, the invention provides a method for producing eukaryotic cells having multiple copies of the product gene comprising transfecting eukaryotic cells with the DNA construct described above (where the selectable gene is an amplifiable gene), growing the cells in a selective medium comprising an amplifying agent for a sufficient time for amplification to occur, and selecting cells having multiple copies of the product gene. Preferably transfection of the cells is achieved using electroporation.

After transfection of the host cells, most of the transfectants fail to exhibit the selectable phenotype characteristic of the protein encoded by the selectable gene, but surprisingly a small proportion of the transfectants do exhibit the selectable phenotype, and among those transfectants, the majority are found to express high levels of the desired product encoded by the product gene. Thus, the invention provides an improved method for the selection of recombinant host cells expressing high levels of a desired product, which method is useful with a wide variety of eukaryotic host cells and avoids the problems inherent in existing cell selection technology.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D illustrate schematically various DNA constructs encompassed by the instant invention. The large arrows represent the selectable gene and the product gene, the V formed by the dashed lines shows the region of the precursor RNA internal to the 5' splice donor site (SD) and 3' splice acceptor site (SA) that is excised from vectors that contain a functional SD. The transcriptional regulatory region, selectable gene, product gene and transcriptional termination site are depicted in Figure 1A. Figure 1B depicts the DNA constructs of Example 1. The various splice donor sequences are depicted, *i.e.*, wild type ras splice donor sequence (WT ras), mutant ras splice donor sequence (MUTANT ras) and non-functional splice donor sequence (▲GT). The probes used for Northern blot analysis in Example 1 are shown in Figure 1B. Figure 1C depicts the DNA constructs of Example 2 and Figure 1D depicts the DNA construct of Example 3 used for expression of anti-IgE V_H.

Figure 2 depicts schematically the control DNA construct used in Example 1.

Figures 3A-Q depict the nucleotide sequence (SEQ ID NO: 1) of the DHFR/intron-(WT ras SD)-tPA expression vector of Example 1.

Figure 4 is a bar graph which shows the number of colonies that form in selective medium after electroporation of linearized duplicate miniprep DNA's prepared in parallel from the three vectors shown in Figure 1B (*i.e.* with wild type ras splice donor sequence [WT ras], mutant ras splice donor sequence [MUTANT ras] and non-functional splice donor sequence [▲GT]) and from the control vector that has DHFR under control of SV40 promoter and tPA under control of CMV promoter (see Figure 2). Cells were selected in nucleoside free medium and counted with an automated colony counter.

Figures 5A-C are bar graphs depicting expression of tPA from stable pools and clones generated from the vectors shown in Figure 1B. In Figure 5A greater than 100 clones from each vector transfection were mixed, plated in 24 well plates, and assayed by tPA ELISA at "saturation". In Figure 5B, twenty clones chosen at random derived from each of the vectors were assayed by tPA ELISA at "saturation". In Figure 5C, the pools mentioned in Figure 5A (except the ▲GT pool) were exposed to 200nM Mtx to select for DHFR amplification and then pooled and assayed for tPA expression.

Figures 6A-P depict the nucleotide sequence (SEQ ID NO: 2) of the DHFR/intron-(WT ras SD)-TNFr-IgG expression vector of Example 2.

Figures 7A-B are bar graphs depicting expression of TNFr-IgG using dicistronic or control vectors (see Example 2). Vectors containing TNFr-IgG (but otherwise identical to those described for tPA expression in Example 1) were constructed (see Figure 1C), introduced into dp12.CHO cells by electroporation, pooled, and assayed for product expression before (Figure 7A) and after (Figure 7B) being subjected to amplification in 200nM Mtx.

Figure 8 depicts schematically the DNA construct used for expression of the V_L of anti-IgE in Example 3.

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Figures 9A-O depict the nucleotide sequence (SEQ ID NO: 3) of the anti-IgE V_H expression vector of Example 3.

Figures 10A-Q depict the nucleotide sequence (SEQ ID NO: 4) of the anti-IgE V_L expression vector of Example 3.

Figure 11 is a bar graph depicting anti-IgE expression in Example 3. Heavy (V_H) and light (V_L) chain expression vectors were constructed, co-electroporated into CHO cells, clones were selected and assayed for antibody expression. Additionally, pools were established and assessed with regard to expression before and after Mtx selection at 200nM and 1μM.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions:

The "DNA construct" disclosed herein comprises a non-naturally occurring DNA molecule which can either be provided as an isolate or integrated in another DNA molecule *e.g.* in an expression vector or the chromosome of an eukaryotic host cell.

The term "selectable gene" as used herein refers to a DNA that encodes a selectable marker necessary for the growth or survival of a host cell under the particular cell culture conditions chosen. Accordingly, a host cell that is transformed with a selectable gene will be capable of growth or survival under certain cell culture conditions wherein a non-transfected host cell is not capable of growth or survival. Typically, a selectable gene will confer resistance to a drug or compensate for a metabolic or catabolic defect in the host cell. Examples of selectable genes are provided in the following table. See also Kaufman, Methods in Enzymology, 185: 537-566 (1990), for a review of these.

TABLE 1

Selectable Genes and their Selection Agents

Selection Agent	Selectable Gene
Methotrexate	Dihydrofolate reductase
Cadmium	Metallothionein
PALA	CAD
Xyl-A-or adenosine and 2'-deoxycoformycin	Adenosine deaminase
Adenine, azaserine, and coformycin	Adenylate deaminase
6-Azauridine, pyrazofuran	UMP Synthetase
Mycophenolic acid	IMP 5'-dehydrogenase

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Mycophenolic acid with limiting xanthine	Xanthine-guanine phosphoribosyltransferase
Hypoxanthine, aminopterin, and thymidine (HAT)	Mutant HGPRTase or mutant thymidine kinase
5-Fluorodeoxyuridine	Thymidylate synthetase
Multiple drugs *e.g.* adriamycin, vincristine or colchicine	P-glycoprotein 170
Aphidicolin	Ribonucleotide reductase
Methionine sulfoximine	Glutamine synthetase
β-Aspartyl hydroxamate or Albizziin	Asparagine synthetase
Canavanine	Arginosuccinate synthetase
α-Difluoromethylornithine	Ornithine decarboxylase
Compactin	HMG-CoA reductase
Tunicamycin	*N*-Acetylglucosaminyl transferase
Borrelidin	Threonyl-tRNA synthetase
Ouabain	Na^+K^+-ATPase

The preferred selectable gene is an amplifiable gene. As used herein, the term "amplifiable gene" refers to a gene which is amplified (*i.e.* additional copies of the gene are generated which survive in intrachromosomal or extrachromosomal form) under certain conditions. The amplifiable gene usually encodes an enzyme (*i.e.* an amplifiable marker) which is required for growth of eukaryotic cells under those conditions. For example, the gene may encode DHFR which is amplified when a host cell transformed therewith is grown in Mtx. According to Kaufman, the selectable genes in Table 1 above can also be considered amplifiable genes. An example of a selectable gene which is generally not considered to be an amplifiable gene is the neomycin resistance gene (Cepko *et al.*, *supra*).

As used herein, "selective medium" refers to nutrient solution used for growing eukaryotic cells which have the selectable gene and therefore includes a "selection agent". Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ([MEM], Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ([DMEM], Sigma) are exemplary nutrient solutions. In addition, any of the media described in Ham and Wallace, Meth. Enz., **58**:44 (1979), Barnes and Sato, Anal. Biochem., **102**:255

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(1980), U.S. Patent Nos. 4,767,704; 4,657,866; 4,927,762; or 4,560,655; WO 90/03430; WO 87/00195; U.S. Patent Re. 30,985; or U.S. Patent No. 5,122,469, the disclosures of all of which are incorporated herein by reference, may be used as culture media. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The preferred nutrient solution comprises fetal bovine serum.

The term "selection agent" refers to a substance that interferes with the growth or survival of a host cell that is deficient in a particular selectable gene. Examples of selection agents are presented in Table 1 above. The selection agent preferably comprises an "amplifying agent" which is defined for purposes herein as an agent for amplifying copies of the amplifiable gene, such as Mtx if the amplifiable gene is DHFR. See Table 1 for examples of amplifying agents.

As used herein, the term "transcriptional initiation site" refers to the nucleic acid in the DNA construct corresponding to the first nucleic acid incorporated into the primary transcript, *i.e.*, the mRNA precursor, which site is generally provided at, or adjacent to, the 5' end of the DNA construct.

The term "transcriptional termination site" refers to a sequence of DNA, normally represented at the 3' end of the DNA construct, that causes RNA polymerase to terminate transcription.

As used herein, "transcriptional regulatory region" refers to a region of the DNA construct that regulates transcription of the selectable gene and the product gene. The transcriptional regulatory region normally refers to a promoter sequence (*i.e.* a region of DNA involved in binding of RNA polymerase to initiate transcription) which can be constitutive or inducible and, optionally, an enhancer (*i.e.* a *cis*-acting DNA element, usually from about 10-300 bp, that acts on a promoter to increase its transcription).

As used herein, "product gene" refers to DNA that encodes a desired protein or polypeptide product. Any product gene that is capable of expression in a host cell may be used, although the methods of the invention are particularly suited for obtaining high-level expression of a product gene that is not also a selectable or amplifiable gene. Accordingly, the protein or polypeptide encoded by a product gene typically will be one that is not necessary for the growth or survival of a host cell under the particular cell culture conditions chosen. For example, product genes suitably encode a peptide, or may encode a polypeptide sequence of

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amino acids for which the chain length is sufficient to produce higher levels of tertiary and/or quaternary structure.

Examples of bacterial polypeptides or proteins include, *e.g.*, alkaline phosphatase and β-lactamase. Examples of mammalian polypeptides or proteins include molecules such as renin; a growth hormone, including human growth hormone, and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; alpha-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA); bombesin; thrombin; hemopoietic growth factor; tumor necrosis factor-alpha and -beta; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-1-alpha); a serum albumin such as human serum albumin; mullerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial protein, such as beta-lactamase; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; integrin; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-β; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF-alpha and TGF-beta, including TGF-β1, TGF-β2, TGF-β3, TGF-β4, or TGF-β5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding proteins; CD proteins such as CD-3, CD-4, CD-8, and CD-19; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), *e.g.*, M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.*, IL-1 to IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport proteins; homing receptors; addressins; regulatory proteins; antibodies; chimeric proteins such as immunoadhesins and fragments of any of the above-listed polypeptides.

The product gene preferably does not consist of an anti-sense sequence for inhibiting the expression of a gene present in the host. Preferred proteins herein are therapeutic proteins such as TGF-β, TGF-α, PDGF, EGF, FGF, IGF-I, DNase, plasminogen activators such as t-PA, clotting factors such as tissue factor and factor VIII, hormones such as relaxin and insulin, cytokines such as IFN-γ, chimeric proteins such as TNF receptor IgG immunoadhesin (TNFr-IgG) or antibodies such as anti-IgE.

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The term "intron" as used herein refers to a nucleotide sequence present within the transcribed region of a gene or within a messenger RNA precursor, which nucleotide sequence is capable of being excised, or spliced, from the messenger RNA precursor by a host cell prior to translation. Introns suitable for use in the present invention are suitably prepared by any of several methods that are well known in the art, such as purification from a naturally occurring nucleic acid or *de novo* synthesis. The introns present in many naturally occurring eukaryotic genes have been identified and characterized. Mount, Nuc. Acids Res., **10**:459 (1982). Artificial introns comprising functional splice sites also have been described. Winey *et al.*, Mol. Cell Biol., **9**:329 (1989); Gatermann *et al.*, Mol. Cell Biol., **9**:1526 (1989). Introns may be obtained from naturally occurring nucleic acids, for example, by digestion of a naturally occurring nucleic acid with a suitable restriction endonuclease, or by PCR cloning using primers complementary to sequences at the 5' and 3' ends of the intron. Alternatively, introns of defined sequence and length may be prepared synthetically using various methods in organic chemistry. Narang *et al.*, Meth. Enzymol., **68**:90 (1979); Caruthers *et al.*, Meth. Enzymol., **154**:287 (1985); Froehler *et al.*, Nuc. Acids Res., **14**:5399 (1986).

As used herein "splice donor site" or "SD" refers to the DNA sequence immediately surrounding the exon-intron boundary at the 5' end of the intron, where the "exon" comprises the nucleic acid 5' to the intron. Many splice donor sites have been characterized and Ohshima *et al.*, J. Mol. Biol., **195**:247-259 (1987) provides a review of these. An "efficient splice donor sequence" refers to a nucleic acid sequence encoding a splice donor site wherein the efficiency of splicing of messenger RNA precursors having the splice donor sequence is between about 80 to 99% and preferably 90 to 95% as determined by quantitative PCR. Examples of efficient splice donor sequences include the wild type (WT) ras splice donor sequence and the GAC:GTAAGT sequence of Example 3. Other efficient splice donor sequences can be readily selected using the techniques for measuring the efficiency of splicing disclosed herein.

The terms "PCR" and "polymerase chain reaction" as used herein refer to the *in vitro* amplification method described in US Patent No. 4,683,195 (issued July 28, 1987). In general, the PCR method involves repeated cycles of primer extension synthesis, using two DNA primers capable of hybridizing preferentially to a template nucleic acid comprising the nucleotide sequence to be amplified. The PCR method can be used to clone specific DNA sequences from total genomic DNA, cDNA transcribed from cellular RNA, viral or plasmid DNAs. Wang & Mark, in PCR Protocols, pp.70-75 (Academic Press, 1990); Scharf, in PCR Protocols, pp. 84-98; Kawasaki & Wang, in PCR Technology, pp. 89-97 (Stockton Press, 1989). Reverse transcription-polymerase chain reaction (RT-PCR) can be used to analyze RNA samples containing mixtures of spliced and unspliced mRNA transcripts. Fluorescently tagged primers designed to span the intron are used to

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amplify both spliced and unspliced targets. The resultant amplification products are then separated by gel electrophoresis and quantitated by measuring the fluorescent emission of the appropriate band(s). A comparison is made to determine the amount of spliced and unspliced transcripts present in the RNA sample.

One preferred splice donor sequence is a "consensus splice donor sequence". The nucleotide sequences surrounding intron splice sites, which sequences are evolutionarily highly conserved, are referred to as "consensus splice donor sequences". In the mRNAs of higher eukaryotes, the 5' splice site occurs within the consensus sequence AG:GUAAGU (wherein the colon denotes the site of cleavage and ligation). In the mRNAs of yeast, the 5' splice site is bounded by the consensus sequence :GUAUGU. Padgett, *et al.*, Ann. Rev. Biochem., 55:1119 (1986).

The expression "splice acceptor site" or "SA" refers to the sequence immediately surrounding the intron-exon boundary at the 3' end of the intron, where the "exon" comprises the nucleic acid 3' to the intron. Many splice acceptor sites have been characterized and Ohshima *et al.*, J. Mol. Biol., **195**:247-259 (1987) provides a review of these. The preferred splice acceptor site is an efficient splice acceptor site which refers to a nucleic acid sequence encoding a splice acceptor site wherein the efficiency of splicing of messenger RNA precursors having the splice acceptor site is between about 80 to 99% and preferably 90 to 95% as determined by quantitative PCR. The splice acceptor site may comprise a consensus sequence. In the mRNAs of higher eukaryotes, the 3' splice acceptor site occurs within the consensus sequence $(U/C)_{11}$NCAG:G. In the mRNAs of yeast, the 3' acceptor splice site is bounded by the consensus sequence (C/U)AG:. Padgett, *et al.*, *supra*.

As used herein "culturing for sufficient time to allow amplification to occur" refers to the act of physically culturing the eukaryotic host cells which have been transformed with the DNA construct in cell culture media containing the amplifying agent, until the copy number of the amplifiable gene (and preferably also the copy number of the product gene) in the host cells has increased relative to the transformed cells prior to this culturing.

The term "expression" as used herein refers to transcription or translation occurring within a host cell. The level of expression of a product gene in a host cell may be determined on the basis of either the amount of corresponding mRNA that is present in the cell or the amount of the protein encoded by the product gene that is produced by the cell. For example, mRNA transcribed from a product gene is desirably quantitated by northern hybridization. Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, pp. 7.3-7.57 (Cold Spring Harbor Laboratory Press, 1989). Protein encoded by a product gene can be quantitated either by assaying for the biological activity of the protein or by employing assays that are independent of such activity, such as western blotting or radioimmunoassay using antibodies that are capable of reacting with the protein. Sambrook,

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et al., Molecular Cloning: A Laboratory Manual, pp. 18.1-18.88 (Cold Spring Harbor Laboratory Press, 1989).

Modes for Carrying Out the Invention

Methods and compositions are provided for enhancing the stability and/or copy number of a transcribed sequence in order to allow for elevated levels of a RNA sequence of interest. In general, the methods of the present invention involve transfecting a eukaryotic host cell with an expression vector comprising both a product gene encoding a desired polypeptide and a selectable gene (preferably an amplifiable gene).

Selectable genes and product genes may be obtained from genomic DNA, cDNA transcribed from cellular RNA, or by *in vitro* synthesis. For example, libraries are screened with probes (such as antibodies or oligonucleotides of about 20-80 bases) designed to identify the selectable gene or the product gene (or the protein(s) encoded thereby). Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the selectable gene or product gene is to use PCR methodology as described in section 14 of Sambrook *et al.*, *supra*.

A preferred method of practicing this invention is to use carefully selected oligonucleotide sequences to screen cDNA libraries from various tissues known to contain the selectable gene or product gene. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized.

The oligonucleotide generally is labeled such that it can be detected upon hybridization to DNA in the library being screened. The preferred method of labeling is to use ^{32}P- labeled ATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labeling.

Sometimes, the DNA encoding the selectable gene and product gene is preceded by DNA encoding a signal sequence having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the expression vector, or it may be a part of the selectable gene or product gene that is inserted into the expression vector. If a heterologous signal sequence is used, it preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. For yeast secretion the native signal sequence may be substituted by, *e.g.*, the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders, the latter described in U.S. Pat. No. 5,010,182 issued 23 April 1991), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression the native signal sequence

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of the protein of interest is satisfactory, although other mammalian signal sequences may be suitable, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders, for example, the herpes simplex gD signal. The DNA for such precursor region is ligated in reading frame to the selectable gene or product gene.

As shown in Figure 1A, the selectable gene is generally provided at the 5' end of the DNA construct and this selectable gene is followed by the product gene. Therefore, the full length (non-spiced) message will contain DHFR as the first open reading frame and will therefore generate DHFR protein to allow selection of stable transfectants. The full length message is not expected to generate appreciable amounts of the protein of interest as the second AUG in a dicistronic message is an inefficient initiator of translation in mammalian cells (Kozak, J. Cell Biol., **115**: 887-903 [1991]).

The selectable gene is positioned within an intron. Introns are noncoding nucleotide sequences, normally present within many eukaryotic genes, which are removed from newly transcribed mRNA precursors in a multiple-step process collectively referred to as splicing.

A single mechanism is thought to be responsible for the splicing of mRNA precursors in mammalian, plant, and yeast cells. In general, the process of splicing requires that the 5' and 3' ends of the intron be correctly cleaved and the resulting ends of the mRNA be accurately joined, such that a mature mRNA having the proper reading frame for protein synthesis is produced. Analysis of a variety of naturally occurring and synthetically constructed mutant genes has shown that nucleotide changes at many of the positions within the consensus sequences at the 5' and 3' splice sites have the effect of reducing or abolishing the synthesis of mature mRNA. Sharp, Science, **235**:766 (1987); Padgett, *et al.*, Ann. Rev. Biochem., 55:1119 (1986); Green, Ann. Rev. Genet., **20**:671 (1986). Mutational studies also have shown that RNA secondary structures involving splicing sites can affect the efficiency of splicing. Solnick, Cell, **43**:667 (1985); Konarska, *et al.*, Cell, **42**:165 (1985).

The length of the intron may also affect the efficiency of splicing. By making deletion mutations of different sizes within the large intron of the rabbit beta-globin gene, Wieringa, *et al.* determined that the minimum intron length necessary for correct splicing is about 69 nucleotides. Cell, **37**:915 (1984). Similar studies of the intron of the adenovirus E1A region have shown that an intron length of about 78 nucleotides allows correct splicing to occur, but at reduced efficiency. Increasing the length of the intron to 91 nucleotides restores normal splicing efficiency, whereas truncating the intron to 63 nucleotides abolishes correct splicing. Ulfendahl, *et al.*, Nuc. Acids Res., **13**:6299 (1985).

To be useful in the invention, the intron must have a length such that splicing of the intron from the mRNA is efficient. The preparation of introns of differing lengths is a routine matter, involving methods well known in the art, such as *de novo* synthesis or *in vitro* deletion

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mutagenesis of an existing intron. Typically, the intron will have a length of at least about 150 nucleotides, since introns which are shorter than this tend to be spliced less efficiently. The upper limit for the length of the intron can be up to 30 kB or more. However, as a general proposition, the intron is generally less than about 10 kB in length.

The intron is modified to contain the selectable gene not normally present within the intron using any of the various known methods for modifying a nucleic acid *in vitro*. Typically, a selectable gene will be introduced into an intron by first cleaving the intron with a restriction endonuclease, and then covalently joining the resulting restriction fragments to the selectable gene in the correct orientation for host cell expression, for example by ligation with a DNA ligase enzyme.

The DNA construct is dicistronic, *i.e.* the selectable gene and product gene are both under the transcriptional control of a single transcriptional regulatory region. As mentioned above, the transcriptional regulatory region comprises a promoter. Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, J. Biol. Chem., 255:2073 [1980]) or other glycolytic enzymes (Hess *et al.*, J. Adv. Enzyme Reg., 7:149 [1968]; and Holland, Biochemistry, 17:4900 [1978]), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman *et al.*, EP 73,657A. Yeast enhancers also are advantageously used with yeast promoters.

Expression control sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide.

Product gene transcription from vectors in mammalian host cells is controlled by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g. the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the product gene, provided such promoters are compatible with the host cell systems.

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The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. Fiers *et al.*, Nature, 273:113 (1978); Mulligan and Berg, Science, 209:1422-1427 (1980); Pavlakis *et al.*, Proc. Natl. Acad. Sci. USA, 78:7398-7402 (1981). The immediate early promoter of the human cytomegalovirus (CMV) is conveniently obtained as a *Hin*dIII E restriction fragment. Greenaway *et al.*, Gene, 18:355-360 (1982). A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. 4,419,446. A modification of this system is described in U.S. 4,601,978. See also Gray *et al.*, Nature, 295:503-508 (1982) on expressing cDNA encoding immune interferon in monkey cells; , Reyes *et al.*, Nature, 297:598-601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus, Canaani and Berg, Proc. Natl. Acad. Sci. USA, 79:5166-5170 (1982) on expression of the human interferon β1 gene in cultured mouse and rabbit cells, and Gorman *et al.*, Proc. Natl. Acad. Sci. USA, 79:6777-6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

Preferably the transcriptional regulatory region in higher eukaryotes comprises an enhancer sequence. Enhancers are relatively orientation and position independent having been found 5' (Lainins *et al.*, Proc. Natl. Acad. Sci. USA, 78:993 [1981]) and 3' (Lusky *et al.*, Mol. Cell Bio., 3:1108 [1983]) to the transcription unit, within an intron (Banerji *et al.*, Cell, 33:729 [1983]) as well as within the coding sequence itself (Osborne *et al.*, Mol. Cell Bio., 4:1293 [1984]). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer (CMV), the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature, 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the product gene, but is preferably located at a site 5' from the promoter.

The DNA construct has a transcriptional initiation site following the transcriptional regulatory region and a transcriptional termination region following the product gene (see Figure 1A). These sequences are provided in the DNA construct using techniques which are well known in the art.

The DNA construct normally forms part of an expression vector which may have other components such as an origin of replication (*i.e.*, a nucleic acid sequence that enables the vector to replicate in one or more selected host cells) and, if desired, one or more additional selectable gene(s). Construction of suitable vectors containing the desired coding and control sequences employs standard ligation techniques. Isolated plasmids or DNA

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fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

Generally, in cloning vectors the origin of replication is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known. The 2μ plasmid origin of replication is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, *i.e.*, they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

For analysis to confirm correct sequences in plasmids constructed, plasmids from the transformants are prepared, analyzed by restriction, and/or sequenced by the method of Messing *et al.*, Nucleic Acids Res., 9:309 (1981) or by the method of Maxam *et al.*, Methods in Enzymology, 65:499 (1980).

The expression vector having the DNA construct prepared as discussed above is transformed into a eukaryotic host cell. Suitable host cells for cloning or expressing the vectors herein are yeast or higher eukaryote cells.

Eukaryotic microbes such as filamentous fungi or yeast are suitable hosts for vectors containing the product gene. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *S. pombe* [Beach and Nurse, Nature, 290:140 (1981)], *Kluyveromyces lactis* [Louvencourt *et al.*, J. Bacteriol., 737 (1983)], *yarrowia* [EP 402,226], *Pichia pastoris* [EP 183,070], *Trichoderma reesia* [EP 244,234], *Neurospora crassa* [Case *et al.*, Proc. Natl. Acad. Sci. USA, 76:5259-5263 (1979)], and *Aspergillus* hosts such as *A. nidulans* [Ballance *et al.*, Biochem. Biophys. Res. Commun., 112:284-289 (1983); Tilburn *et al.*, Gene, 26:205-221 (1983); Yelton *et al.*, Proc. Natl. Acad. Sci. USA, 81:1470-1474 (1984)] and *A. niger* [Kelly and Hynes, EMBO J., 4:475-479 (1985)].

Suitable host cells for the expression of the product gene are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda*

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(caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosphila melanogaster* (fruitfly), and *Bombyx mori* host cells have been identified. See, e.g., Luckow *et al.*, Bio/Technology, **6**:47-55 (1988); Miller *et al.*, in Genetic Engineering, Setlow, J.K. *et al.*, eds., Vol. 8 (Plenum Publishing, 1986), pp. 277-279; and Maeda *et al.*, Nature, **315**:592-594 (1985). A variety of such viral strains are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain the product gene. During incubation of the plant cell culture with *A. tumefaciens*, the product gene is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the product gene. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker *et al.*, J. Mol. Appl. Gen., **1**:561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA *780* gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. EP 321,196 published 21 June 1989.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years [Tissue Culture, Academic Press, Kruse and Patterson, editors (1973)]. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, J. Gen Virol., **36**:59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, **77**:4216 [1980]); dp12.CHO cells (EP 307,247 published 15 March 1989); mouse sertoli cells (TM4, Mather, Biol. Reprod., **23**:243-251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, Annals N.Y. Acad. Sci., **383**:44-68 [1982]); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

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Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, Gene, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) may be used. General aspects of mammalian cell host system transformations have been described by Axel in U.S. 4,399,216 issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, J. Bact., 130:946 (1977) and Hsiao *et al.*, Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells such as by nuclear injection or by protoplast fusion may also be used.

In the preferred embodiment the DNA is introduced into the host cells using electroporation. See Andreason, J. Tiss. Cult. Meth., 15:56-62 (1993), for a review of electroporation techniques useful for practicing the instantly claimed invention. It was discovered that electroporation techniques for introducing the DNA construct into the host cells were preferable over calcium phosphate precipitation techniques insofar as the latter could cause the DNA to break up and forming concantemers.

The mammalian host cells used to express the product gene herein may be cultured in a variety of media as discussed in the definitions section above. The media contains the selection agent used for selecting transformed host cells which have taken up the DNA construct (either as an intra- or extra-chromosomal element). To achieve selection of the transformed eukaryotic cells, the host cells may be grown in cell culture plates and individual colonies expressing the selectable gene (and thus the product gene) can be isolated and grown in growth medium until the nutrients are depleted. The host cells are then analyzed for transcription and/or transformation as discussed below. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 [1980]), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly ^{32}P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescens, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the

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formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu *et al.*, Am. J. Clin. Path., 75:734-738 (1980).

In the preferred embodiment, the mRNA is analyzed by quantitative PCR (to determine the efficiency of splicing) and protein expression is measured using ELISA as described in Example 1 herein.

The product of interest preferably is recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysates when directly expressed without a secretory signal. When the product gene is expressed in a recombinant cell other than one of human origin, the product of interest is completely free of proteins or polypeptides of human origin. However, it is necessary to purify the product of interest from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to the product of interest. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. The product of interest thereafter is purified from contaminant soluble proteins and polypeptides, for example, by fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel electrophoresis using, for example, Sephadex G-75; chromatography on plasminogen columns to bind the product of interest and protein A Sepharose columns to remove contaminants such as IgG.

The following examples are offered by way of illustration only and are not intended to limit the invention in any manner. All patent and literature references cited herein are expressly incorporated by reference.

EXAMPLE 1

tPA production using the dicistronic expression vectors

It was sought to increase the level of homogeneity with regard to expression levels of stable clones by expressing a selectable marker (such as DHFR) and the protein of interest from a single promoter. These vectors divert most of the transcript to product expression while linking it at a fixed ratio to DHFR expression via differential splicing.

Vectors were constructed which were derived from the vector pRK (Suva *et al.*, Science, 237:893-896 [1987]) which contains an intron between the cytomegalovirus immediate early promoter (CMV) and the cDNA that encodes

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the polypeptide of interest. The intron of pRK is 139 nucleotides in length, has a splice donor site derived from cytomegalovirus immediate early gene (CMVIE), and a splice acceptor site from an IgG heavy chain variable region (V_H) gene (Eaton *et al.*, Biochem., **25**:8343 [1986]).

DHFR/intron vectors were constructed by inserting an EcoRV linker into the BSTX1 site present in the intron of pRK7. An 830 base-pair fragment containing a mouse DHFR coding fragment was inserted to obtain DHFR intron expression vectors which differ only in the sequence that comprises the splice donor site. Those sequences were altered by overlapping PCR mutagenesis to obtain sequences that match splice donor sites found between exons 3 and 4 of normal and mutant Ras genes. PCR was also used to destroy the splice donor site.

A mouse DHFR cDNA fragment (Simonsen *et al.*, Proc. Natl. Acad. Sci. USA, **80**:2495-2499 [1983]) was inserted into the intron of this vector 59 nucleotides downstream of the splice donor site. The splice donor site of this vector was altered by mutagenesis to change the ratio of spliced to non-spliced message in transfected cells. It has previously been shown that a single nucleotide change (G to A) converted a relatively efficient splice donor site found in the normal ras gene into an inefficient splice site (Cohen *et al.*, Nature, **334**:119-124 [1988]). This effect has been demonstrated in the context of the ras gene and confirmed when these sequences were transferred to human growth hormone constructs (Cohen *et al.*, Cell, **58**:461-472 [1989]). Additionally, a non functional 5' splice site (GT to CA) was constructed as a control (ΔGT). A polylinker was inserted 35 nucleotides downstream of the 3' splice site to accept the cDNA of interest. A vector containing tPA (Pennica *et al.*, Nature, **301**:214-221 [1983]) was linearized downstream of the polyadenylation site before it was introduced into CHO cells (Potter *et al.*, Proc. Natl. Acad. Sci. USA, **81**:7161 [1984]).

Plasmid DNA's that contained DHFR/intron, tPA and (a) wild type ras (WT ras), *i.e.* Figure 3 (SEQ ID NO: 1), (b) mutant ras, or (c) non-functional splice donor site (ΔGT) were introduced into CHO DHFR minus cells by electroporation. The intron vectors were each linearized downstream of the polyadenylation site by restriction endonuclease treatment. The control vector was linearized downstream of the second polyadenylation site. The DNA's were ethanol precipitated after phenol/chloroform extraction and were resuspended in 20μl 1/10 Tris EDTA. Then, 10μg of DNA was incubated with 10^7 CHO.dp12 cells (EP 307,247 published 15 March 1989) in 1 ml of PBS on ice for 10 min. before electroporation at 400 volts and 330μf using a BRL Cell Porator.

Cells were returned to ice for 10 min. before being plated into non-selective medium. After 24 hours cells were fed nucleoside-free medium to select for stable DHFR+ clones which were pooled. The pooled DHFR+ clones were lysed and mRNA's were prepared.

To prepare the mRNA, RNA was extracted from 5 x 10^7 cells which were grown from pools of more than 200 clones derived from the stable

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transfection of the three vectors, the essential construction of which is shown in Figure 1B and from non-transfected CHO cells. RNA was purified over oligo-DT cellulase (Collaborative Biomedical Products). 10μg of mRNA was then subjected to Northern blotting which involved running the mRNA on a 1.2% agarose, 6.6% formaldehyde gel, and transferring it to a nylon filter (Stratagene Duralon-UV membrane), prehybridized, probed and washed according to the manufacturer's instructions.

The filter was probed sequentially using probes (shown in Figure 1B) that would detect (a) the full length message, (b) both full length and spliced message, or (c) beta actin. Probing with the long probe showed that the vector that contains the efficient splice donor site (*i.e.* WT ras) generates predominately a mRNA of the size predicted for the spliced product while the other two vectors gave rise primarily to a mRNA that corresponds in size to non-spliced message. The DHFR probe detected only full length message and demonstrated that the WT ras splice donor derived vector generates very little full length message with which to confer a DHFR positive phenotype.

Figure 4 shows the number of DHFR positive colonies obtained after duplicate electroporations with the three intron vectors described above and from a conventional vector that has a CMV promoter driving tPA and a SV40 promoter driving DHFR (see Figure 2). The increase in colony number parallels the increase in full length message that accumulates with the modification of the splice donor sites. The conventional vector efficiently generates colonies and does not vary significantly from the ΔGT construct.

The level of tPA expression was determined by seeding cells in 1 ml of F12:DMEM (50:50, with 5% FBS) in 24 well dishes to near confluency. Growth of the cells continued until the media was exhausted. Media was then assayed by ELISA for tPA production. Briefly, anti-tPA antibody was coated onto the wells of an ELISA microtiter plate, media samples were added to the wells followed by washing. Binding of the antigen (tPA) was then quantified using horse radish peroxidase (HRPO) labelled anti-tPA antibody.

Figure 5A depicts the titers of secreted tPA protein after pooling the clones of each group shown in Figure 4. While the number of colonies increased with a weakening of splice donor function, the inverse was seen with respect to tPA expression. The expression levels are consistent with the RNA products that are observed; as more of the dicistronic message is spliced an increased amount of message will contain tPA as the first open reading frame resulting in increased tPA expression. A mutation of GT to CA in the splice donor site results in an abundance of DHFR positive colonies which express undetectable levels of tPA, possibly resulting from inefficient utilization of the second AUG. Importantly, Figure 5A also shows that expression levels obtained from one of the dicistronic vectors (with WT ras SD) was about threefold higher than that obtained with the control vector containing a CMV promoter/enhancer driving tPA, SV40

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promoter/enhancer controlling DHFR and SV40 polyadenylation signals controlling the expression of tPA and DHFR.

Additionally, the homogeneity of expression in the pools was investigated. Figure 5B shows that all 20 clones generated by the WT ras splice donor site derived dicistronic vectors express detectable levels of tPA while only 4 of 20 clones generated by the control vector express tPA. None of the clones transfected with the non-splicing (ΔGT) vector expressed tPA levels detectable by ELISA. This finding is consistent with previous observations that relatively few clones generated by conventional vectors make useful levels of protein.

Expression of tPA was increased following methotrexate amplification of pools. Figure 5C shows that 2 of the dicistronic vector derived pools (*i.e.* with WT ras and MUTANT ras SD sites) increased in expression markedly (8.4 and 7.7 fold), while the pool generated by the conventional vector increased only slightly (2.8 fold) when each was subjected to 200 nM Mtx. An overall increase of 9 fold was obtained using the best dicistronic (WT ras SD) versus the conventional vector following amplification. Growth of the highest expressing amplified pool in nutrient rich production medium yielded titers of 4.2 μg/ml tPA.

It was shown that manipulation of the splice donor sequence alters the ratio of spliced to full length message and the number of colonies that form in selective medium. It was also shown that dicistronic expression vectors generate clones that express high levels of recombinant proteins. Surprisingly, it was possible to isolate high expressors which had the efficient WT ras splice donor site by selection for DHFR$^-$ cells despite the efficiency with which the DHFR gene was spliced from the RNA precursors formed in these cells.

EXAMPLE 2

TNFr-IgG production using the dicistronic expression vectors

To prove the general applicability of this approach, a second product was evaluated in the dicistronic vector system containing, as the DNA of interest, an immunoadhesin (TNFr-IgG) capable of binding tumor necrosis factor (TNF) (Ashkenazi *et al.*, Proc. Natl. Acad. Sci. USA, **88**:10535-10539 [1991]). The experiments described in Example 1 above were essentially repeated except that the product gene encoded the immunoadhesin TNFr-IgG. Plasmid DNA's that contained a TNFr-IgG cDNA and (a) WT ras, *i.e.* Figure 6 (SEQ ID NO: 2), (b) mutant ras or (c) nonfunctional splice donor site (ΔGT) were introduced into the dp12.CHO cells as discussed for Example 1. See Figure 1C for an illustration of the DNA constructs.

It was discovered that the number of DHFR positive colonies generated by three of these vectors was similar to that seen with the tPA constructs. Expression of TNFr-IgG also paralleled that seen with the tPA constructs (Figure 7A). Amplification of pools from two of the constructs showed a marked increase in expression of immunoadhesin (9.6 and 6.8 fold) (Figure

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7B). The best of these amplified pools expressed 9.5 μg/ml when grown in nutrient rich production medium.

Thus, it was again shown that dicistronic expression vectors generate clones that express high levels of recombinant proteins. Furthermore, contrary to expectations, it was discovered that isolation of high product expressing host DHFR- cells was possible using an efficient splice donor site (i.e. the WT ras splice donor site).

EXAMPLE 3

Antibody production using a dicistronic expression vector

The usefulness of this system for antibody expression was evaluated by testing production of an antibody directed against IgE (Presta *et al.*, Journal of Immunology, 151:2623-2632 [1993]). Further, the flexibility of the system with regard to transcription initiation was tested by replacing the CMV promoter/enhancer present in the previous vectors with the promoter/ enhancer derived from the early region of SV40 virus (Griffin, B., Structure and Genomic Organization of SV40 and Polyoma Virus, In J. Tooze [Ed] DNA Tumor Viruses, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The heavy chain of the antibody was inserted downstream of DHFR as described in the earlier tPA and TNFr-IgG constructs. Additionally, a new splice donor site sequence (GAC:GTAAGT) was engineered into the vector which matches the consensus splice donor site more closely than did the splice donor sites present in the vectors tested in Examples 1 and 2. The resultant expression vector is shown in Figures 1D and 9.

It was discovered that this vector produced fewer colonies than the vectors previously tested, and produced predominantly a spliced RNA product. A second vector was constructed to have the light chain of the antibody under control of the SV40 promoter/enhancer and poly-A and the hygromycin B resistance gene under control of the CMV promoter/enhancer and SV40 poly-A. These vectors were linearized at unique HpaI sites downstream of the poly-A signal, mixed at a ratio of light chain vector to heavy chain vector of 10:3 and electroporated into CHO cells using an optimized protocol (as discussed in Examples 1 and 2).

Figure 11 shows the levels of antibody expressed by clones and pools after selection in hygromycin B followed by selection for DHFR expression. All 20 of the clones analyzed expressed high levels of antibody when grown in rich medium and varied from one another by only a factor of four. A pool of antibody producing clones was generated and assayed shortly after it was established. That pool was grown continuously for 6 weeks without a significant decrease in productivity demonstrating that its stability was sufficient to generate gram quantities of protein from its large scale culture.

The pool was subjected to methotrexate amplification at 200nM and 1μM and achieved a greater than 2 fold increase in antibody titer. The 1μM Mtx resistant pool achieved a titer of 41 mg/L when grown under optimal conditions in suspension culture.

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The structure of the expressed antibody was examined. Proteins expressed by the 200nM methotrexate resistant pool and by a well characterized expression clone generated by conventional vectors (Presta *et al.* [1993], *supra*) were metabolically labeled with S^{35} cysteine and methionine. In particular, confluent 35mm plates of cells were metabolically labeled with 50μCi each S-35 methionine and S-35 cysteine (Amersham) in serum free cysteine and methionine free F12:DMEM. After one hour, nutrient rich production media was added and labeled proteins were allowed to "chase" into the medium for six more hours. Proteins were run on a 12% SDS/PAGE gel (NOVEX) non-reduced or following reduction with B-mercaptoethanol. Dried gels were exposed to film for 16 hours. CHO control cells were also labeled.

The majority of the antibody protein is secreted with a molecular weight of about 155 kilodaltons, consistent with a properly disulfide-linked antibody molecule with 2 light and 2 heavy chains. Upon reduction the molecular weight shifts to 2 approximately equally abundant proteins of 22.5 and 55 kilodaltons. The protein generated from the pool is indistinguishable from the antibody produced by the well characterized expression clone, with no apparent increase of free heavy or light chain expressed by the pool.

CONCLUSION

The efficient expression system described herein utilizes vectors consisting of promoter/enhancer elements followed by an intron containing the selectable marker coding sequence, followed by the cDNA of interest and a polyadenylation signal.

Several splice donor site sequences were tested for their effect on colony number and expression of the cDNA of interest. A non-functional splice donor site, splice donor sites found in an intron between exons 3 and 4 of mutant (mutant ras) and normal (WT ras) forms of the Harvey Ras gene and another efficient SD site (see Example 3) were used. The vectors were designed to direct expression of dicistronic primary transcripts. Within a transfected cell some of the transcripts remain full length while the remainder are spliced to excise the DHFR coding sequence. When the splice donor site is weakened or destroyed an increase in colony number is observed.

Expression levels show the inverse pattern, with the most efficient splice donor sites generating the highest levels of tPA, TNFr immunoadhesin or anti-IgE V_H.

The homogeneity of expression of clones generated by the ras splice donor site intron DHFR vectors was compared to clones generated from a conventional vector with a separate promoter/enhancer and polyadenylation signal for each DHFR and tPA. The DHFR intron vector gives rise to colonies that are much more homogeneous with regard to expression than those generated by the conventional vector. Non-expressing clones derived from the conventional vector may be the result of breaks in the tPA or

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TNFr-IgG domain of the plasmid during integration into the genome or the result of methylation of promoter elements (Busslinger *et al.*, Cell, 34:197-206 [1983]; Watt *et al.*, Genes and Development, 2:1136-1143 [1988]) driving tPA or TNFr-IgG expression. Promoter silencing by methylation or breaks in the DHFR-intron vectors would very likely render them incapable of conferring a DHFR positive phenotype.

It was found that pools generated by the DHFR-intron vectors could be amplified in methotrexate and would increase in expression by a factor of 8.4 (tPA), or 9.8 (TNFr-IgG). Pools from conventional vectors increased by only 2.8 and 3.0 fold for tPA and TNFr-IgG when amplified similarly. Amplified pools resulted in 9 fold higher tPA levels and 15 fold higher TNFr-IgG levels when compared to the conventional vector amplified pools.

Without being limited to any theory, the increase in expression of methotrexate resistant pools derived from the dicistronic vectors is likely due to the transcriptional linkage of DHFR and the product; when cells are selected for increased DHFR expression they consistently over-express product. Conventional approaches lack selectable marker and cDNA expression linkage and therefore methotrexate amplification often generates DHFR overexpression without the concomitant increase in product expression.

A further increase of 4 and 6.3 fold in expression were obtained when amplified tPA and TNFr-IgG pools were transferred from the media used for the selections and amplifications to a nutrient rich production medium.

In Example 3, the expression vector had a splice donor site that more closely matches the consensus splice donor sequence and had the heavy chain of a humanized anti-IgE antibody inserted downstream. This vector was linearized and co-electroporated with a second linearized vector that expresses the hygromycin resistance gene and the light chain of the antibody each under the control of its own promoter/enhancer and poly-A signals. An excess of light chain expression vector over the heavy chain dicistronic expression vector was used to bias in favor of light chain expression. Clones and a pool were generated after hygromycin B and DHFR selections. The clones were found to express relatively consistent, high levels of antibody, as did the pool. The 1μM pool achieved a titer of 41mg/L when grown under optimal conditions in suspension culture.

The anti-IgE antibody was assessed by metabolic labeling followed by SDS/PAGE under reducing and non reducing conditions and found to be indistinguishable from the protein expressed by a highly characterized clonal cell line. Of particular importance is the finding that no free light chain is observed in the pool relative to the clone.

A stable expression system for CHO cells has been developed that produces high levels of recombinant proteins rapidly and with less effort than that required by other expression systems. The vector system generates stable clones that express consistently high levels thereby reducing the number of clones that must be screened to obtain a highly productive clonal line. Alternatively, pools have been used to conveniently generate moderate to high levels of protein. This approach

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may be particularly useful when a number of related proteins are to be expressed and compared.

Without being limited to this theory, it is possible the vectors that have very efficient splice donor sites generate very productive clones because so little transcript remains non spliced that only integration events that lead to the generation of high levels of RNA produce enough DHFR protein to give rise to colonies in selective medium. The high level of spliced message from such clones is then translated into abundant amounts of the protein of interest. Pools of clones made concurrently by introducing conventional vectors expressed lower levels of protein, and were unstable with regard to long term expression, and expression could not be appreciably increased when the cells were subjected to methotrexate amplification.

The system developed herein is versatile in that it allows high levels of single and multiple subunit polypeptides to be rapidly generated from clones or pools of stable transfectants. This expression system combines the advantages of transient expression systems (rapid and labor non intensive generation of research amounts of protein) with the concurrent development of highly productive stable production cell lines.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: GENENTECH, INC.

(ii) TITLE OF INVENTION: METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
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(B) STREET: 460 Point San Bruno Blvd
(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: patin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/286740
(B) FILING DATE: 05-AUG-1994

(viii) ATTORNEY/AGENT INFORMATION:
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(B) REGISTRATION NUMBER: 00,000
(C) REFERENCE/DOCKET NUMBER: 798PCT

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(B) TELEFAX: 415/952-9881
(C) TELEX: 910/371-7168

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7360 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TACGAATTAA 7360

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6889 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6557 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC 250

CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT 300

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TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA ATACTGTCCT 5700

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GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA 6500

TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC 6550

GAATTAA 6557

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7305 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

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ATTAA 7305

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CLAIMS

1. A DNA construct comprising a transcriptional initiation site, a transcriptional termination site, a selectable gene, a product gene provided 3' to the selectable gene, a transcriptional regulatory region regulating transcription of both the selectable gene and the product gene, the selectable gene being positioned within an intron having a splice donor site 5' of the intron, which splice donor site regulates expression of the product gene using the transcriptional regulatory region.

2. The DNA construct of claim 1 wherein the splice donor site comprises an efficient splice donor sequence.

3. The DNA construct of claim 2 wherein the splice donor site comprises a consensus splice donor sequence.

4. The DNA construct of claim 2 wherein the splice donor site comprises the sequence GACGTAAGT.

5. The DNA construct of claim 1 wherein the selectable gene is an amplifiable gene.

6. The DNA construct of claim 5 wherein the amplifiable gene is DHFR.

7. The DNA construct of claim 1 wherein the transcriptional regulatory region comprises a promoter and an enhancer.

8. A vector comprising the DNA construct of claim 1.

9. The vector of claim 8 wherein the selectable gene of the DNA construct is an amplifiable gene.

10. The vector of claim 8 that is capable of replication in a eukaryotic host.

11. A eukaryotic host cell comprising the vector of claim 10.

12. A eukaryotic host cell comprising the DNA construct of claim 5.

13. The host cell of claim 11 wherein the vector is introduced into the host cell by electroporation.

14. A eukaryotic host cell comprising the DNA construct of claim 1 integrated into a chromosome of the host cell.

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15. The host cell of claim 14 that is a mammalian cell.

16. A method for producing a product of interest comprising culturing the host cell of claim 11 so as to express the product gene and recovering the product from the host cell culture.

17. The method of claim 16 further comprising recovering the product from the culture medium.

18. The method of claim 16 wherein the selectable gene is an amplifiable gene and the splice donor site comprises an efficient splice donor sequence.

19. A method for producing a product of interest comprising culturing the host cell of claim 12 so as to express the product gene in a selective medium comprising an amplifying agent for sufficient time to allow amplification to occur, and recovering the product.

20. A method for producing eukaryotic cells having multiple copies of a product gene comprising transforming eukaryotic cells with the DNA construct of claim 5, growing the cells in a selective medium comprising an amplifying agent for a sufficient time for amplification to occur, and selecting cells having multiple copies of the product gene.

21. The method of claim 20 further comprising recovering from the selected cells the product of interest.

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FIG. 1A

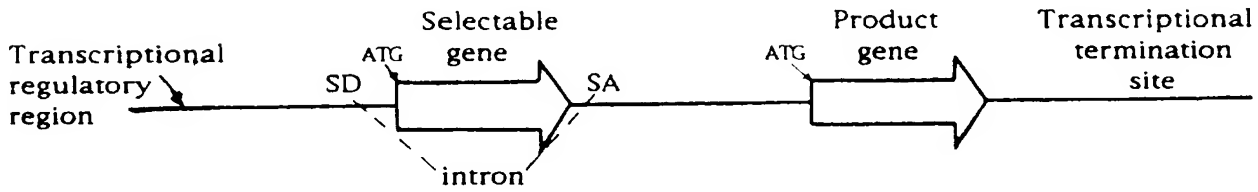
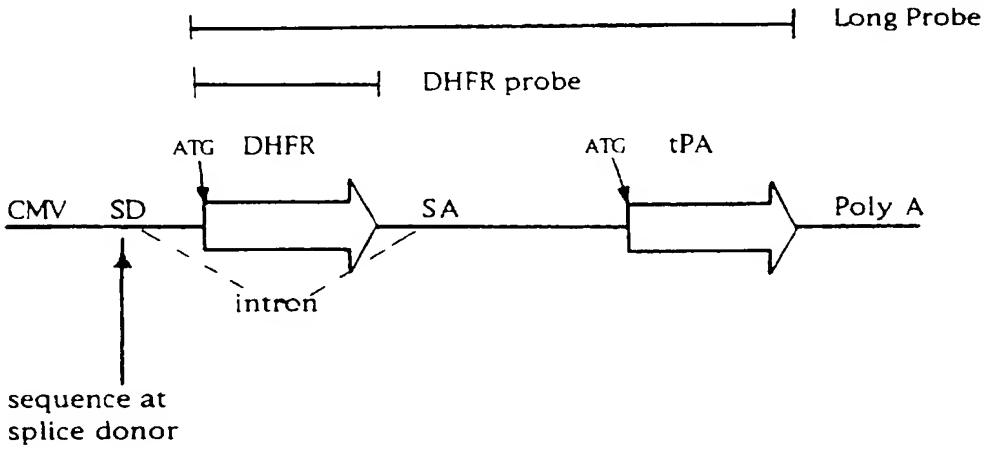
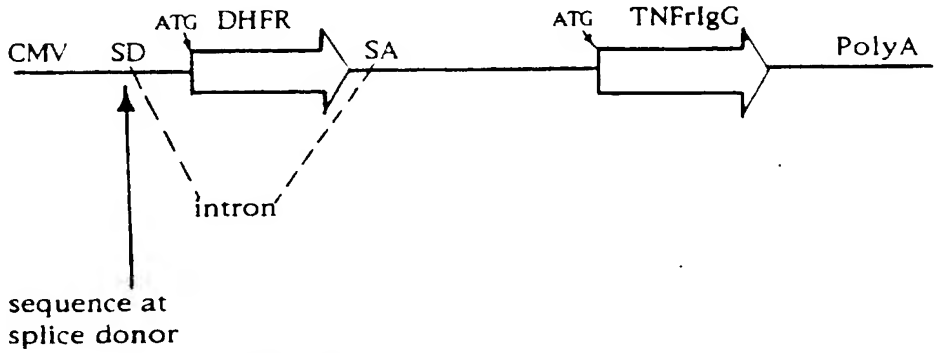


FIG. 1B



a) WT ras (efficient SD)
b) MUTANT ras (less efficient SD)
c) ▲GT (inefficient SD)

FIG. 1C



a) WT ras
b) MUTANT ras
c) ▲GT

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FIG. 1D

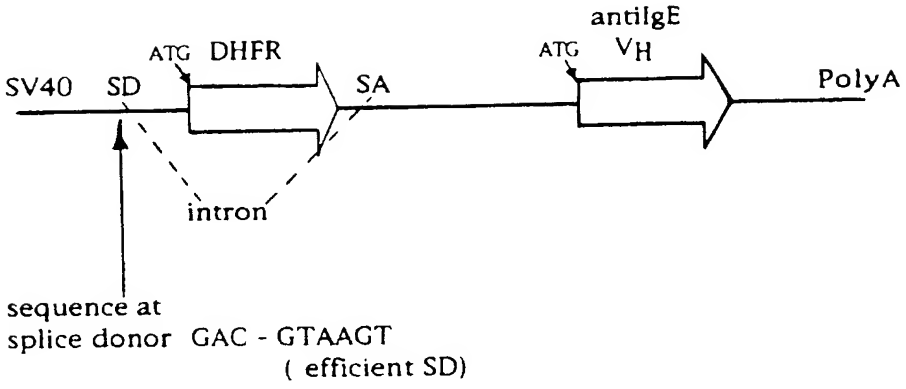
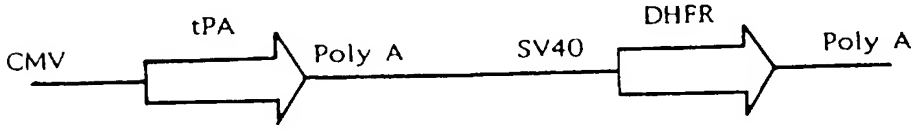


FIG. 2



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BNSDOCID <WO__9604391A1_I_>

FIG. 3A

```
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        sstI
        sacI
        hgiJII
        hgiAI/aspHI
        ecl136II
        bsp1286                                                                    thaI
        bsiHKAI                                                                    fnuDII/mvnI
        bmyI                          rmaI     tru9I                               bstUI
        banII                         maeI     mseI                                bsh1236I
      taqI                           speI     aseI/asnI/vspI                 bslI          aciI maeIII
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    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                     scrFI
                     mvaI
                     ecoRII
                     dsaV
                  aciI
                bglI bstNI                              maeII
               sau96I                                   hinlI/acyI
               haeIII/palI     aciI                     ahaII/bsaHI
               asuI  apyI[dcm+]                aciI     aatII          maeII          maeIII
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      maeII                                                                        maeII
     hinlI/acyI                                                                    hinlI/acyI
     ahaII/bsaHI                                    rsaI                  rsaI     ahaII/bsaHI
     aatII                                bglI      csp6I          ndeI   csp6I    aatII
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           scrFI
           mvaI
           ecoRII
       aciI                                                                          nlaIII
     bglI  dsaV                                                                styI
    sau96I bstNI          rsaI                                    maeII           ncoI
    haeIII/palI           csp6I                             rsaI  snaBI          dsaI hphI  aciI
    asuI   apyI[dcm+]   bsrI  nlaIII                       csp6I  bsaAI          bsaJI   sfaNI
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BNSDOCID <WO 9604391A1 >

SUBSTITUTE SHEET (RULE 26)

FIG. 3B

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                                                                                                                    maeII
                                                                                                                  hinlI/acyI                nlaIV
                                rsaI                                  pleI                                        ahaII/bsaHI               hgiCI
                                csp6I                          aciI   hinfI                    bsmAI               aatII                     banI
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                                                                                                                                    aluI
                                                                                                                                   sstI
                                                                                                                                   sacI
                                                                                                                                   hgiJII
                                                                                                                                   hgiAI/aspHI
                                                                                                                                   ecl136II
                                                                                                                                   bsp1286
                                                                                                                                   bsiHKAI
                                                                                                                          rsaI     bmyI
                                        maeIII     aciI        hgaI        aciI          csp6I      mnlI                  banII
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                                                                                                                                  haeIII/palI
                                                                                                                                 mcrI
                                                                                                                                 eagI/xmaIII/eclXI
                                                                                                                                 eaeI
                                                                                                                                 cfrI
                                                                                                                                fnu4HI
                                                                                                                                aciI
                                                                                                                               thaI
                                    esp3I                                                                                      fnuDII/mvnI
                               scrFI                                                          sau96I          sacII/sstII
                               mvaI bsmAI                                                          avaII           nspBII
                               ecoRII                                                         asuI            kspI  scrFI
                               dsaV                                                          nlaIV            dsaI  nciI
                               bstNI  hinlI/acyI                                                 scrFI         bglI bslI  mspI
                               apyI[dcm+]                                                        nciI     sau3AI mnlI bstUI
                          sau3AI gsuI/bpmI                                                       mspI     mboI/ndeII[dam-] hpaII
                          mboI/ndeII[dam-]                                              mboII    hpaII    dpnI[dam+] bsaJI dsaV
                          dpnI[dam+]  hgaI  fokI                                        bpuAI    dsaV     dpnII[dam-] bsh1236I
                          dpnII[dam-] ahaII/bsaHI                    mnlI     bbsI     cauII    alwI[dam-] aciI  cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

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FIG. 3C

```
                tfiI
             aciI
            thaI hinfI
            fnuDII/mvnI                                  aciI                                                  fnu4HI
            bstUI                                                                                              bbvI
            bsh1236I                                  rsaI                                                  nspBII          taqI
                                                      csp6I   scfI          mnlI                            aciI       nlaIII
701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT
    AACCTTGCGC CTAAGGGGCA CGGTTCTCAC GACATTCATG GCGGATATCT CGCTATTCTC CTAAAATAGG GGCGACGGTA GTACCAAGCT GGTAACTTGA

                                                                                                           thaI
                                                                                                           fnuDII/mvnI
                                                                                                           bstUI
                                                                                                           bsh1236I
                                                                                                        mluI
                                                                                  bsrBI                 aflIII      rsaI
                        pflMI                                bsmAI              aciI          xmnI              csp6I
    sfaNI               bslI                                 bsaI          mnlI   ddeI asp700            scaI
801 GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC
    CGTAGCAGCG GCACAGGGTT TTATACCCCT AACCGTTCTT GCCTCTGGAT GGGACGGGAG GCGAGTCCTT GCGCAAGTTC ATGAAGGTTT CTTACTGGTG

                                                                  scrFI
                                                                  mvaI
                                                                  ecoRII
                                                                  dsaV
            eco57I                                                bstNI                                 tfiI        tru9I          tru9I
           mboII                    tfiI                          apyI[dcm+]                         mboII taqI    mseI           mseI
          earI/ksp632I              hinfI                                                                                          aseI/asnI/vspI
        mnlI                      alwNI    hphI                  sexAI                 ddeI    hinfI     ahaIII/draI
901 AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT
    TTGGAGAAGT CACCTTCCAT TTGTCTTAGA CCACTAATAC CCATCCTTTT GGACCAAGAG GTAAGGACTC TTCTTAGCTG GAAATTTCCT GTCTTAATTA

                                        aluI
                                        sstI
                                        sacI
                                        hgiJII
                                        hgiAI/aspHI
                                        ecl136II
                                        bsp1286
                                        bsiHKAI
                                        bmyI                                                  tru9I                mspI
                                        banII                                          sfaNI  mseI                 hpaII
          ddeI                    bslI    mnlI                  bstXI       fokI       aflII/bfrI          bsaWI
1001 ATAGTTCTCA GTAGAGAACT CAAAGAACCA CCACGAGGAG CTCATTTTCT TGCCAAAAGT TTGGATGATG CCTTAAGACT TATTGAACAA CCGGAATTGG
     TATCAAGAGT CATCTCTTGA GTTTCTTGGT GGTGCTCCTC GAGTAAAAGA ACGGTTTTCA AACCTACTAC GGAATTCTGA ATAACTTGTT GGCCTTAACC
```

FIG. 3D

```
                                                                               haeIII/palI
                                                                              haeI
                                                       scrFI          scrFI                        nlaIII
                                                       mvaI                 mvaI                            sau3AI
                                                       ecoRII         ecoRII                    mboI/ndeII[dam-]
                                                       dsaV   tfiI    dsaV    pleI              dpnI[dam+]
                                                       bstNI     nlaIII     bstNI     ddeI                  dpnII[dam-]
             accI  nlaIII             mnlI             apyI[dcm+]   hinfI   apyI[dcm+]    hinfI    maeIII  alwI[dam-]
1101 CAAGTAAAGT AGACATGGTT TGGATAGTCG GAGGCAGTTC TGTTTACCAG GAAGCCATGA ATCAACCAGG CCACCTTAGA CTCTTTGTGA CAAGGATCAT
     GTTCATTTCA TCTGTACCAA ACCTATCAGC CTCCGTCAAG ACAAATGGTC CTTCGGTACT TAGTTGGTCC GGTGGAATCT GAGAAACACT GTTCCTAGTA

                                                                                          mnlI
                                                                                     hinlI/acyI         scrFI
                                                                                     ahaII/bsaHI        mvaI
                                                                                  scrFI           ecoRII
                                                                                  mvaI    ecoNI         dsaV
                                                                                  ecoRII      sau96I
                                                                                  dsaV        avaII
                         maeII                                                    bstNI   bslI      asuI    mnlI
                       aflIII                                                     apyI[dcm+]      mnlI  bstNI
         apoI       maeIII                                       mnlI            bsaJI hgaI      ddeI   apyI[dcm+]
1201 GCAGGAATTT GAAAGTGACA CGTTTTTCCC AGAAATTGAT TTGGGGAAAT ATAAACCTCT CCCAGAATAC CCAGGCGTCC TCTCTGAGGT CCAGGAGGAA
     CGTCCTTAAA CTTTCACTGT GCAAAAAGGG TCTTTAACTA AACCCCTTTA TATTTGGAGA GGGTCTTATG GGTCCGCAGG AGAGACTCCA GGTCCTCCTT

                                                                                                   ppu10I
                                                             sfaNI                                 nsiI/avaIII
         sfaNI                 accI     mboII            mboII                        mnlI     aluI
1301 AAAGGCATCA AGTATAAGTT TGAAGTCTAC GAGAAGAAAG ACTAACAGGA AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA
     TTTCCGTAGT TCATATTCAA ACTTCAGATG CTCTTCTTTC TGATTGTCCT TCTACGAAAG TTCAAGAGAC GAGGGGAGGA TTTCGATACG TAAAAATATT

                                                         fnu4HI
        nlaIII                                           aciI
       styI                                             thaI
       ncoI                                             fnuDII/mvnI tru9I
       dsaI                           styI              bstUI       mseI                                      maeIII
       bsaJI                          bsaJI             bsh1236I  aseI/asnI/vspI                             hphI
1401 GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA
     CTGGTACCCT GAAAACGACC GAAATCTGGG GGAACCGAAG CAATCTTGCG CCGATGTTAA TTATGTATTG GAATACATAG TATGTGTATC TAAATCCACT
```


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FIG. 3E

```
                                                           scrFI
                                                           mvaI
                                                           ecoRII                           bspMI
                                                           dsaV                            scfI
                                                           bstNI                                pstI
                                                           apyI[dcm+]      mnlI       aluI      bsgI                tru9I
                                                         gsuI/bpmI         bsaJI     hindIII   fnu4HI               mseI
        scfI        fokI                             maeIII     hincII/hindII    ddeI         bbvI           apoI
1501 CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA GGTCAACTGC ACCTCGGTTC TAAGCTTGGG CTGCAGGTCG CCGTGAATTT
     GTGATATCTT ATTGTAGGTG AAACGGAAAG AGAGGTGTCC ACAGTGAGGT CCAGTTGACG TGGAGCCAAG ATTCGAACCC GACGTCCAGC GGCACTTAAA

                                                 hgiJII                                                    scrFI
                                                 bsp1286                                                   mvaI
                                                 bmyI             fnu4HI                                   ecoRII
                                 sfaNI         mnlI               bbvI              mboII                  dsaV
                                fokI      mboII  banII          fnu4HI             bpuAI                   bstNI
          hgaI               nlaIII       earI/ksp632I          bbvI               bbsI                    apyI[dcm+] nlaIII
1601 AAGGGACGCT GTGAAGCAAT CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC CAGGAAATCC
     TTCCCTGCGA CACTTCGTTA GTACCTACGT TACTTCTCTC CCGAGACGAC ACACGACGAC GACACACCTC GTCAGAAGCA AAGCGGGTCG GTCCTTTAGG

                                                  scfI
                                 sau3AI           pstI
                                 mboI/ndeII[dam-] bsgI                                                          hgiAI/aspHI
                         nlaIV   dpnI[dam+]     sau3AI                                                   hinPI  bsp1286
                       mnlI      bstYI/xhoII    mboI/ndeII[dam-]                                     fnu4HI     bsiHKAI
          tfiI     mboII         bglII          dpnI[dam+]                                           bbvI       bmyI
          hinfI    earI/ksp632I  dpnII[dam-]    dpnII[dam-]                                     nlaIII   hhaI/cfoI
1701 ATGCCCGATT CAGAAGAGGA GCCAGATCTT ACCAAGTGAT CTGCAGAGAT GAAAAAACGC AGATGATATA CCAGCAACAT CAGTCATGGC TGCGCCCTGT
     TACGGGCTAA GTCTTCTCCT CGGTCTAGAA TGGTTCACTA GACGTCTCTA CTTTTTTGCG TCTACTATAT GGTCGTTGTA GTCAGTACCG ACGCGGGACA

                   scrFI
                   nciI
                   mspI
                   hpaII                                       alwNI
                   dsaV                         bsp1286       ddeI                 fnu4HI   styI
       ddeI        cauII    sspI                bmyI         draIII                bbvI     bsaJI
1801 GCTCAGAAGC AACCGGGTGG AATATTGCTG GTGCAACAGT GGCAGGGCAC AGTGCCACTC AGTGCCTGTC AAAAGTTGCA GCGAGCCAAG GTGTTTCAAC
     CGAGTCTTCG TTGGCCCACC TTATAACGAC CACGTTGTCA CCGTCCCGTG TCACGGTGAG TCACGGACAG TTTTCAACGT CGCTCGGTTC CACAAAGTTG
```


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FIG. 3F

```
                                                                                              pmlI
                                                                                              eco72I
                                                                                           sau96I
                                                                                           asuI maeII
                                                                                        scrFI
                                                                                        mvaI haeIII/palI
                                                                                        ecoRII
                                                                                        dsaV
                                                                                        bstNI   bsaAI
     bspMI                                                                              bsaJI   bbrPI
  nlaIV         sau96I                                                                  apyI[dcm+]
  hgiCI         haeIII/palI
  banI          asuI   rsaI                      bsp1286
 bsp1286       eco0109I/draII                    bmyI
 bmyI          alwNI    csp6I ddeI            bsrI     bcgI
1901 GGGGGCACCT GCCAGCAGGC CCTGTACTTC TCAGATTTCG TGTGCCAGTG CCCCGAAGGA TTTGCTGGGA AGTGCTGTGA AATAGATACC AGGGCCACGT
     CCCCCGTGGA CGGTCGTCCG GGACATGAAG AGTCTAAAGC ACACGGTCAC GGGGCTTCCT AAACGACCCT TCACGACACT TTATCTATGG TCCCGGTGCA

          scrFI                                   hinPI
          mvaI                                         hhaI/cfoI
          ecoRII                                  nlaIV   hgiAI/aspHI
          dsaV                                    narI    bsp1286             thaI
          bstNI                 pmlI  hgiAI/aspHI     kasI    bsiHKAI              fnuDII/mvnI
          bsaJI                 eco72I                hinlI/acyI                   bstUI sau96I
       sau96I               bsaAI bsp1286         hgiCI   bmyI              hinPI  haeIII/palI
       avaII                bbrPI bsiHKAI         haeII   apaLI/snoI        hhaI/cfoI
       asuI     sfaNI   scfI   bsp1286   bmyI    aciI     banI    alw44I/snoI     fnu4HI    asuI       bslI
     mnlI  apyI[dcm+] aluI  bmyI maeII     nspBII      ahaII/bsaHI     bsrI       bbvI bsh1236I        bslI
2001 GCTACGAGGA CCAGGGCATC AGCTACAGGG GCACGTGGAG CACAGCGGAG AGTGGCGCCG AGTGCACCAA CTGGAACAGC AGCGCGTTGG CCCAGAAGCC
     CGATGCTCCT GGTCCCGTAG TCGATGTCCC CGTGCACCTC GTGTCGCCTC TCACCGCGGC TCACGTGGTT GACCTTGTCG TCGCGCAACC GGGTCTTCGG

                                   scrFI
                                   mvaI
                                   ecoRII
                                   dsaV                                 pleI      scrFI
                                   bstNI                                   bsmAI       mvaI
                                   bsaJI                                 taqI[dam-]    ecoRII
                      hinlI/acyI   apyI[dcm+]                           sau3AI hinfI   dsaV
        aciI  haeIII/palI         haeIII/palI          scfI             mboI/ndeII[dam-]  bstNI
   nspBII     haeI  hgaI bstXI    sau96I         pstI             dpnI[dam+]      apyI[dcm+]     tru9I
 scfI aciI    mnlI  ahaII/bsaHI   asuI     nlaIV       bsgI         dpnII[dam-]     bsaJI        maeII mseI
2101 CTACAGCGGG CGGAGGCCAG ACGCCATCAG GCTGGGCCTG GGGAACCACA ACTACTGCAG AAACCCAGAT CGAGACTCAA AGCCCTGGTG CTACGTCTTT
     GATGTCGCCC GCCTCCGGTC TGCGGTAGTC CGACCCGGAC CCCTTGGTGT TGATGACGTC TTTGGGTCTA GCTCTGAGTT TCGGGACCAC GATGCAGAAA
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FIG. 3G

```
                                        fnu4HI
                                        bbvI
                                aluI    scfI
                              rsaI      pstI                    mnlI      maeIII                          dsaI
                    aciI      csp6I ddeI bsgI                  ddeI      alwNI                           bsaJI
2201 AAGGCGGGGA AGTACAGCTC AGAGTTCTGC AGCACCCCTG CCTGCTCTGA GGGAAACAGT GACTGCTACT TTGGGAATGG GTCAGCCTAC CGTGGCACGC
     TTCCGCCCCT TCATGTCGAG TCTCAAGACG TCGTGGGGAC GGACGAGACT CCCTTTGTCA CTGACGATGA AACCCTTACC CAGTCGGATG GCACCGTGCG
                                                                                                    scrFI
                                                                                                    pflMI
                                                                                                    mvaI
                                                                                                    ecoRII
                                                                                                    dsaV
                                                                                                    bstNI
                                                                                                    bslI
                                                                                                    apyI[dcm+] haeIII/palI
                                                                                                    bsp1286       sau96I
                                                                                                    bmyI alwNI    asuI
                                                                                                    bsrI  bsaJI   bsrI
                                                sau3AI
                                                mboI/ndeII[dam-]
                                                dpnI[dam+]
                                                dpnII[dam-]
                                                alwI[dam-]
                                                nlaIV
                                                hgiCI
                                                banI  maeII
                                                scrFI
                                                pmlI
                                                ecoRII
                                                dsaV
                                                bstNI     eco72I
                                                pflMI     bsaAI
                                                apyI[dcm+]       mboII                                  rsaI
                                                                                                        csp6I      bsp1286
                                                fokI  bslI bsaJI     bbrPI  eco57I  aciI       maeII   scaI       bmyI
2401 CCTGGGCAAA CATAATTACT GCCGGAATCC TGATGGGGAT GCCAAGCCCT GGTGCCACGT GCTGAAGAAC CGCAGGCTGA CGTGGGAGTA CTGTGATGTG
     GGACCCGTTT GTATTAATGA CGGCCTTAGG ACTACCCCTA CGGTTCGGGA CCACGGTGCA CGACTTCTTG GCGTCCGACT GCACCCTCAT GACACTACAC
                                                                                                    scrFI
                                                                                                    mvaI
                                                                                                    ecoRII
                                                                                                    dsaV
                                                                                                    bstNI
                                                                                                    apyI[dcm+]
                                                                                                    bslI bsaJI     fnu4HI
                                                                                                    mnlI   bslI    bbvI
                                                                                                    mnlI    sapI
                                                                                                    sfaNI     mnlI
                                                                                                    csp6I      mnlI
                                                                                                    haeIII/palI
                                                                                                    bspMI
                                                                                                    mnlI
                                                                                                    banII
                                                                                                    bmyI
                                                                                                    bsp1286
                                                                                                    hgiJII
                                                                                                    earI/ksp632I
                                                                                                    mboII
                                                                                                    ddeI
                                                                                                    rsaI
                                                                                                    ddeI
                                                                                                    aciI
                                                                                                    fnu4HI  bsmAI
2501 CCCTCCTGCT CCACCTGCGG CCTGAGACAG TACAGCCAGC CTCAGTTTCG CATCAAAGGA GGGCTCTTCG CCGACATCGC CTCCCACCCC TGGCAGGCTG
     GGGAGGACGA GGTGGACGCC GGACTCTGTC ATGTCGGTCG GAGTCAAAGC GTAGTTTCCT CCCGAGAAGC GGCTGTAGCG GAGGGTGGGG ACCGTCCGAC
```

FIG. 3H

```
                                    mspI                                                                                scrFI
                                    hpaII                                                                               mvaI
                                    bslI                                                                                ecoRII
                                    scrFI                                                                               dsaV
                                    nciI          nlaIV                                                                 bstNI
                                    dsaV      aciI                                  aluI          tfiI          aciI    apyI[dcm+]
                bslI          mnlI  cauII   bsrBI     bslI aciI                    alwNI          hinfI         fnu4HI
2601 CCATCTTTGC CAAGCACAGG AGGTCGCCCG GAGAGCGGTT CCTGTGCGGG GGCATACTCA TCAGCTCCTG CTGGATTCTC TCTGCCGCCC ACTGCTTCCA
     GGTAGAAACG GTTCGTGTCC TCCAGCGGGC CTCTCGCCAA GGACACGCCC CCGTATGAGT AGTCGAGGAC GACCTAAGAG AGACGGCGGG TGACGAAGGT

                                                                  scrFI
                                                                  mvaI
                                                                  ecoRII
                                                                  dsaV
                                                                  bstNI
                                                                  apyI[dcm+]
                                                  scrFI       bslI
                                    sau3AI        nciI        bsaJI
                                    mboI/ndeII[dam-]  mspI    sau96I
                                    dpnI[dam+]    hpaII   nlaIV
                                    dpnII[dam-]   dsaV    avaII          mnlI
        mnlI    aciI    bslI    hphI[dam-]        cauII   asuI       mnlI          apoI        taqI
2701 GGAGAGGTTT CCGCCCCACC ACCTGACGGT GATCTTGGGC AGAACATACC GGGTGGTCCC TGGCGAGGAG GAGCAGAAAT TTGAAGTCGA AAAATACATT
     CCTCTCCAAA GGCGGGGTGG TGGACTGCCA CTAGAACCCG TCTTGTATGG CCCACCAGGG ACCGCTCCTC CTCGTCTTTA AACTTCAGCT TTTTATGTAA

                                                  scfI                                  scrFI
                                                  pstI                                  mvaI
                                                 fnu4HI                                 ecoRII
                                                 bbvI pvuII                             dsaV
                                               fnu4HI fnu4HI                            bstNI                 aciI
                                               bbvI   bbvI                        bspl286       fnu4HI   sau96I
              ecoRI                          hinPI bsgI aluI        tfiI      nspBII  bsaJI      bbvI     avaII
              apoI taqI                      hhaI/cfoI nspBII       hinfI     aciI bmyI apyI[dcm+]       asuI
2801 GTCCATAAGG AATTCGATGA TGACACTTAC GACAATGACA TTGCGCTGCT GCAGCTGAAA TCGGATTCGT CCCGCTGTGC CCAGGAGAGC AGCGTGGTCC
     CAGGTATTCC TTAAGCTACT ACTGTGAATG CTGTTACTGT AACGCGACGA CGTCGACTTT AGCCTAAGCA GGGCGACACG GGTCCTCTCG TCGCACCAGG
```

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FIG. 3I

```
                                                                                                aluI
                                                                                                sstI
                                                                                                sacI
                                                                                                hgiJII
                                                                                                hgiAI/aspHI
                                                                                                ecl136II                                haeIII/palI
                                                                                                bsp1286                                 stuI
                                                                                                bsiHKAI                                 haeI
                                                                                                bmyI    mspI                            mnlI
     draIII    bslI bsaJI aciI  bsgI aluI hpaII                                                 banII   hpaII          nlaIII      bsmAI
2901 GCACTGTGTG CCTTCCCCCG GCGGACCTGC AGCTGCCGGA CTGGACGGAG TGTGAGCTCT CCGGCTACGG CAAGCATGAG GCCTTGTCTC CTTTCTATTC
     CGTGACACAC GGAAGGGGGC CGCCTGGACG TCGACGGCCT GACCTGCCTC ACACTCGAGA GGCCGATGCC GTTCGTACTC CGGAACAGAG GAAAGATAAG

                                                                                                fnu4HI
                                                                                              nspBII
       fnu4HI                                                                                 aciI                                                                  nlaIII
       aciI                                                rsaI fokI   fnu4HI                                  tru9I        hphI      nspI
      bsrBI eco57I mnlI  nlaIII                            csp6I    alwNI bbvI                               mseI        maeIII     nspHI        gsuI/bpmI
3001 GGAGCGGCTG AAGGAGGCTC ATGTCAGACT GTACCCATCC AGCCGCTGCA CATCACAACA TTTACTTAAC AGAACAGTCA CCGACAACAT GCTGTGTGCT
     CCTCGCCGAC TTCCTCCGAG TACAGTCTGA CATGGGTAGG TCGGCGACGT GTAGTGTTGT AAATGAATTG TCTTGTCAGT GGCTGTTGTA CGACACACGA

                          sau96I
                          nlaIV
                          haeIII/palI
                          asuI
                         sau96I
                         nlaIV scrFI                                                            scrFI
                         hgiJII                                                                 mvaI
                         eco0109I/draII                                 scrFI                   ecoRII
                         bsp1286                                        mvaI                         dsaV
                         bsp120I                                        ecoRII                  bstNI
                         bmyI  mvaI                                     dsaV          sau96I
                         banII ecoRII                        bstNI                    nlaIV                                 aciI
                        aciI    dsaV                         bsaJI                    haeIII/palI                           fnu4HI
                    fnu4HI     bstNI                  hinlI/acyI        tfiI        asuI apyI[dcm+]                        haeIII/palI
                    aciI asuI  apyI[dcm+]             hgaI     apyI[dcm+]           eco0109I/draII                        eaeI
     bsmAI      bsrBI  apaI bsaJI                     ahaII/bsaHI      hinfI   mnlI  bsaJI                              cfrI   nlaIII
3101 GGAGACACTC GGAGCGGCGG GCCCCAGGCA AACTTGCACG ACGCCTGCCA GGGCGATTCG GGAGGCCCCC TGGTGTGTCT GAACGATGGC CGCATGACTT
     CCTCTGTGAG CCTCGCCGCC CGGGGTCCGT TTGAACGTGC TGCGGACGGT CCCGCTAAGC CCTCCGGGGG ACCACACAGA CTTGCTACCG GCGTACTGAA
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FIG. 3J

```
                                                      scrFI
                                                      nciI
                          scrFI                 mspI
                          mvaI                        hpaII
                          ecoRII                dsaV
                          dsaV                  cauII
                          bstNI                      xmaI/pspAI
                          bsaJI                      smaI
                          apyI[dcm+]                 scrFI
                     nlaIV                           nciI
                     eco0109I/draII            dsaV
                 aluI sau96I                   cauII     rsaI  styI maeIII                    tfiI        nspI
                pvuII asuI                           bsaJI      csp6I bsaJI                rmaI     hinfI       nspHI
        sfaNI   nspBII   haeIII/palI           fokI  avaI     bsp1407I   bstEII            maeI bsrI    maeIII nlaIII
3201 TGGTGGGCAT CATCAGCTGG GGCCTGGGCT GTGGACAGAA GGATGTCCCG GGTGTGTACA CCAAGGTTAC CAACTACCTA GACTGGATTC GTGACAACAT
     ACCACCCGTA GTAGTCGACC CCGGACCCGA CACCTGTCTT CCTACAGGGC CCACACATGT GGTTCCAATG GTTGATGGAT CTGACCTAAG CACTGTTGTA

                                                           mboII
                 scrFI                             aciI earI/ksp632I
                 mvaI                                sau3AI mnlI
                 ecoRII                        mboI/ndeII[dam-]
                 dsaV                          dpnI[dam+]          mboII
                 bstNI            mnlI               dpnII[dam-]   eco57I
          bslI   apyI[dcm+]  pleI                    alwI[dam-]  mboII  bpuAI          hinPI
     mcrI maeIII             hinfI                  bstYI/xhoII  mboII   bbsI          hhaI/cfoI   scfI
3301 GCGACCGTGA CCAGGAACAC CCGACTCCTC AAAAGCAAAT GAGATCCCGC CTCTTCTTCT TCAGAAGACA CTGCAAAGGC GCAGTGCTTC TCTACAGACT
     CGCTGGCACT GGTCCTTGTG GGCTGAGGAG TTTTCGTTTA CTCTAGGGCG GAGAAGAAGA AGTCTTCTGT GACGTTTCCG CGTCACGAAG AGATGTCTGA

                                       bsmAI             mboII
       gsuI/bpmI        aciI     aciI  bsaI  scfI   mnlI earI/ksp632I                                        tth111I/aspI
3401 TCTCCAGACC CACCACACCG CAGAAGCGGG ACGAGACCCT ACAGGAGAGG GAAGAGTGCA TTTTCCCAGA TACTTCCCAT TTTGGAAGTT TTCAGGACTT
     AGAGGTCTGG GTGGTGTGGC GTCTTCGCCC TGCTCTGGGA TGTCCTCTCC CTTCTCACGT AAAAGGGTCT ATGAAGGGTA AAACCTTCAA AAGTCCTGAA

                                                                                   scrFI
                                                             scrFI      mvaI
                                                             mvaI bsmI             ecoRII
                                                             ecoRII     dsaV
                                                             dsaV       bstNI
                                      mboII                  bstNI                 bsaJI
                                      bpuAI   bsmI     rmaI gsuI/bpmI   mnlI     bsaJI              ecoRI  taqI
                                      bbsI nlaIII      maeI mnlI apyI[dcm+]  mnlI apyI[dcm+]        apoI   claI/bsp106
3501 GGTCTGATTT CAGGATACTC TGTCAGATGG GAAGACATGA ATGCACACTA GCCTCTCCAG GAATGCCTCC TCCCTGGGCA GAAGTGGGGG GAATTCAATC
     CCAGACTAAA GTCCTATGAG ACAGTCTACC CTTCTGTACT TACGTGTGAT CGGAGAGGTC CTTACGGAGG AGGGACCCGT CTTCACCCCC CTTAAGTTAG
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BNSDOCID <WO 9604391A1 I>

FIG. 3K

```
                styI
              aciI
             fnu4HI   sau96I
             bglI nlaIII
            sfiI ncoI haeIII/palI
            haeIII/palI                    aluI
           eaeI  dsaI asuI               fnu4HI
           cfrI  bsaJI                   bbvI          maeIII              sfaNI  apoI                                  bsmI
3601 GATGGCCGCC ATGGCCCAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA TAAAGCAATA GCATCACAAA TTTCACAAAT AAAGCATTTT TTTCACTGCA
     CTACCGGCGG TACCGGGTTG AACAAATAAC GTCGAATATT ACCAATGTTT ATTTCGTTAT CGTAGTGTTT AAAGTGTTTA TTTCGTAAAA AAAGTGACGT

                                                          sau3AI
                                                          mboI/ndeII[dam-]
                                                          dpnI[dam+]
                                                          dpnII[dam-]
                                                         pvuI/bspCI
                                                         mcrI
                                                        taqI[dam-]  tru9I
                                                      claI/bsp106[dam-]              styI
                                                     sau3AI          mseI        fnu4HI    haeI
                                                     mboI/ndeII[dam-]            bbvI  ncoI
                                                     dpnI[dam+] xmnI         hinPI     dsaI haeIII/palI
          rmaI                                       dpnII[dam-]   aseI/asnI/vspI     bsaJI
          maeI                                  nlaIII alwI[dam-]  asp700      hhaI/cfoI nlaIII           mnlI
3701 TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA TCGATCGGGA ATTAATTCGG CGCAGCACCA TGGCCTGAAA TAACCTCTGA
     AAGATCAACA CCAAACAGGT TTGAGTAGTT ACATAGAATA GTACAGACCT AGCTAGCCCT TAATTAAGCC GCGTCGTGGT ACCGGACTTT ATTGGAGACT

                       rsaI                                                                          nlaIV
                       csp6I                                                                        scrFI
                      nlaIV                                                                         mvaI
                      kpnI                                                                          ecoRII
                      hgiCI                                                                         dsaV
                      banI                          aluI                                            bstNI
                      asp718     mnlI               pvuII                                           apyI[dcm+]
          mnlI        acc65I   ddeI aciI           nspBII                                         bsaJI
3801 AAGAGGAACT TGGTTAGGTA CCTTCTGAGG CGGAAAGAAC CAGCTGTGGA ATGTGTGTCA GTTAGGGTGT GGAAAGTCCC CAGGCTCCCC AGCAGGCAGA
     TTCTCCTTGA ACCAATCCAT GGAAGACTCC GCCTTTCTTG GTCGACACCT TACACACAGT CAATCCCACA CCTTTCAGGG GTCCGAGGGG TCGTCCGTCT
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SUBSTITUTE SHEET (RULE 26)

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FIG. 3L

BNSDOCID <WO___9604391A1_I_>

FIG. 3M

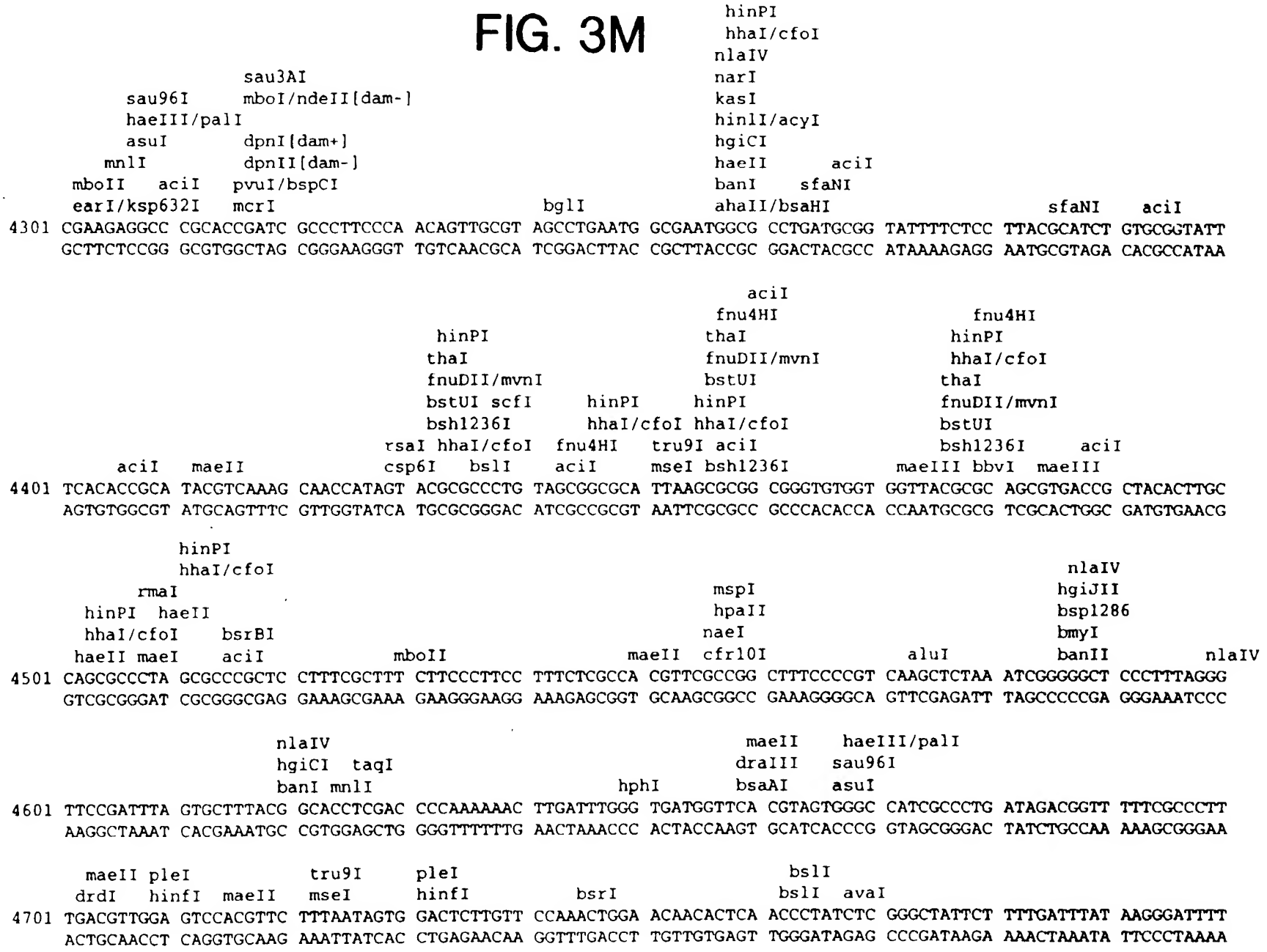
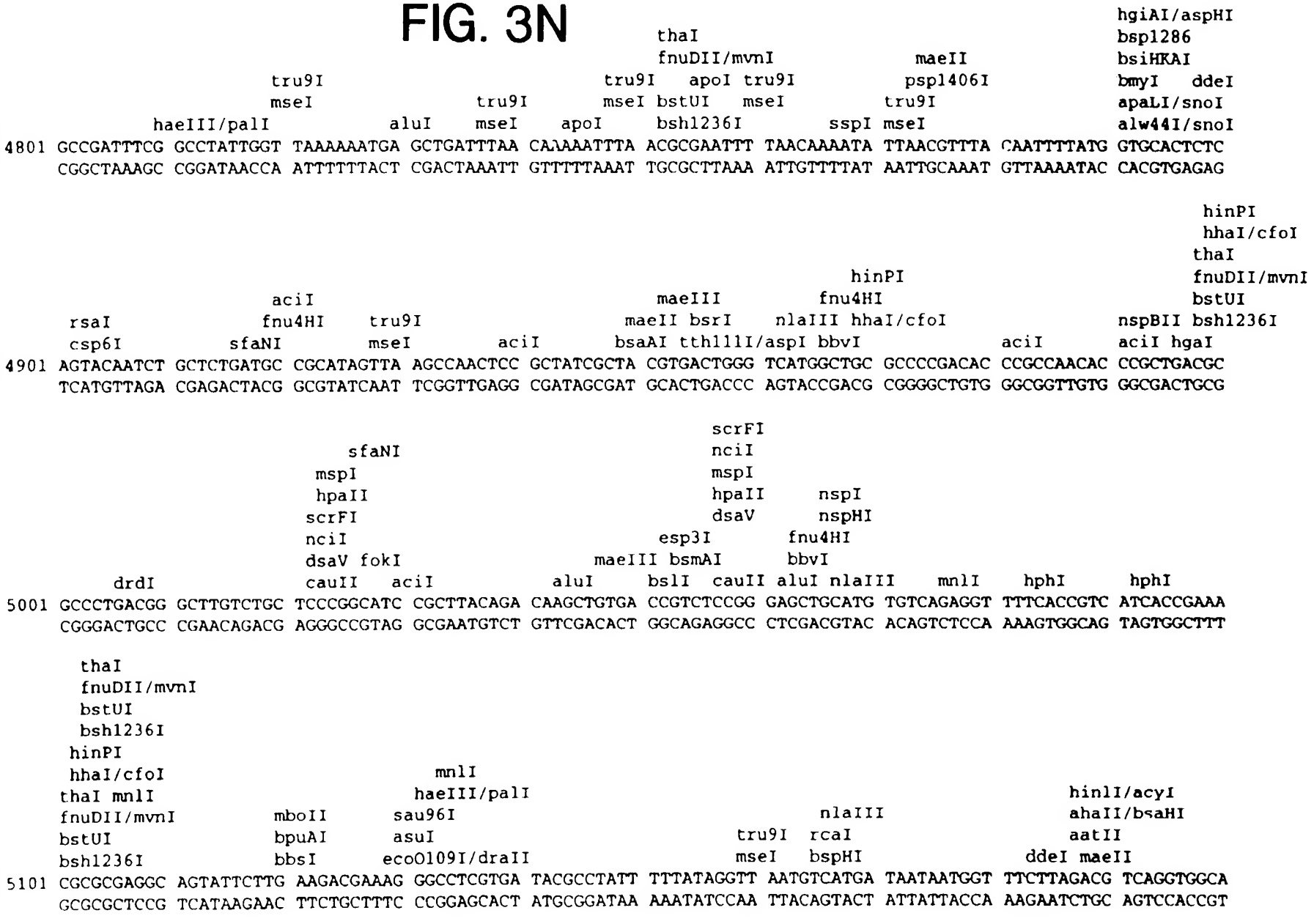


FIG. 3N



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BNSDOCID <WO__9604391A1_I_>

FIG. 3O

```
                                   nlaIV
                                  aciI
                                 thaI
                                 fnuDII/mvnI
                                 bstUI                                                    rcaI
                                 bsh1236I                                                 bspHI
                                hinPI                                                  bsrBI    bsmAI
                                hhaI/cfoI                                              aciI  nlaIII
5201 CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA
     GAAAAGCCCC TTTACACGCG CCTTGGGGAT AAACAAATAA AAAGATTTAT GTAAGTTTAT ACATAGGCGA GTACTCTGTT ATTGGGACTA TTTACGAAGT

                         mboII                                                    fnu4HI
       sspI              earI/ksp632I                                             aciI                                hphI
5301 ATAATATTGA AAAAGGAAGA GTATGAGTAT TCAACATTTC CGTGTCGCCC TTATTCCCTT TTTTGCGGCA TTTTGCCTTC CTGTTTTTGC TCACCCAGAA
     TATTATAACT TTTTCCTTCT CATACTCATA AGTTGTAAAG GCACAGCGGG AATAAGGGAA AAAACGCCGT AAAACGGAAG GACAAAAACG AGTGGGTCTT

                                        hgiAI/aspHI
                                        bsp1286
                                  sau3AI     bsiHKAI                    sau3AI   nspBII    sau3AI
                                  mboI/ndeII[dam-]                      mboI/ndeII[dam-]   mboI/ndeII[dam-]
                                  dpnI[dam+] bmyI                       dpnI[dam+]         dpnI[dam+]
                                  dpnII[dam-]                          bstYI/xhoII         dpnII[dam-]
                              eco57I          apaLI/snoI            bsrI dpnII[dam-]       alwI[dam-]
          hphI           sfaNI  mboII[dam-]   alw44I/snoI  maeIII taqI    alwI[dam-] aciI  bstYI/xhoII
5401 ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC
     TGCGACCACT TTCATTTTCT ACGACTTCTA GTCAACCCAC GTGCTCACCC AATGTAGCTT GACCTAGAGT TGTCGCCATT CTAGGAACTC TCAAAAGCGG

                                                                                  scrFI
                                                                 aciI             nciI
                                                                thaI              mspI
          maeII                                                 fnuDII/mvnI       hpaII
          psp1406I          hgiAI/aspHI                         bstUI             dsaV
          xmnI              bsp1286  tru9I                      bsh1236I        hinII/acyI
          asp700            bsiHKAI  mseI                      hinPI           hgaI cauII                    aciI
        mboII               bmyI   ahaIII/draI                 hhaI/cfoI       ahaII/bsaHI     bcgI  mcrI  fnu4HI
5501 CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT
     GGCTTCTTGC AAAAGGTTAC TACTCGTGAA AATTTCAAGA CGATACACCG CGCCATAATA GGGCACTACT GCGGCCCGTT CTCGTTGAGC CAGCGGCGTA

                                  rsaI
                                  csp6I   bsrI                                                    fnu4HI
             ddeI                 scaI  hphI  maeIII      sfaNI     fokI   nlaIII                bbvI        nlaIII
5601 ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC
     TGTGATAAGA GTCTTACTGA ACCAACTCAT GAGTGGTCAG TGTCTTTTCG TAGAATGCCT ACCGTACTGT CATTCTCTTA ATACGTCACG ACGGTATTGG
```

FIG. 3P

```
                                                 sau96I
                                                 avaII
                                          sau3AI asuI                                                   nlaIII
                 haeIII/palI              mboI/ndeII[dam-]                                            sau3AI maeIII
                eaeI                      dpnI[dam+]                                                  mboI/ndeII[dam-]
                cfrI                      dpnII[dam-]                                                 dpnI[dam+]
               fnu4HI                    pvuI/bspCI                                                   dpnII[dam-]
               aciI                      mcrI   mnlI             aluI    aciI                 nlaIII alwI[dam-]
5701 ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC
     TACTCACTAT TGTGACGCCG GTTGAATGAA GACTGTTGCT AGCCTCCTGG CTTCCTCGAT TGGCGAAAAA ACGTGTTGTA CCCCCTAGTA CATTGAGCGG

                                                                                                       hinPI
              mspI                                                                                     hhaI/cfoI
  sau3AI   nlaIV                                                                                      mstI
  mboI/ndeII[dam-] aluI                                                                               aviII/fspI     bsrI
  dpnI[dam+]    hpaII                                                              fnu4HI          maeII          tru9I
  dpnII[dam-] bsaWI                                             maeIII     sfaNI   bbvI          psp1406I         mseI
5801 TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT GACACCACGA TGCCAGCAGC AATGGCAACA ACGTTGCGCA AACTATTAAC
     AACTAGCAAC CCTTGGCCTC GACTTACTTC GGTATGGTTT GCTGCTCGCA CTGTGGTGCT ACGGTCGTCG TTACCGTTGT TGCAACGCGT TTGATAATTG

                         mspI
                          hpaII                                                                        bglI
                         scrFI                                                                         sau96I
                  aluI   nciI      tru9I      fokI                               sau96I                haeIII/palI
                 rmaI    dsaV      mseI    bsrI          aciI                    avaII        hinPI   asuI     mspI
                 maeI    cauII     aseI/asnI/vspI   mnlI                         asuI         hhaI/cfoI        hpaII
5901 TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC
     ACCGCTTGAT GAATGAGATC GAAGGGCCGT TGTTAATTAT CTGACCTACC TCCGCCTATT TCAACGTCCT GGTGAAGACG CGAGCCGGGA AGGCCGACCG

                          mspI             thaI
                          hpaII            fnuDII/mvnI             haeIII/palI
                         cfr10I            bstUI                  sau96I
                      nlaIV hphI        bsmAI aciI          fnu4HI  nlaIV
                    gsuI/bpmI           bsaI  bsh1236I      bbvI bsrI asuI               mnlI
6001 TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT
     ACCAAATAAC GACTATTTAG ACCTCGGCCA CTCGCACCCA GAGCGCCATA GTAACGTCGT GACCCCGGTC TACCATTCGG GAGGGCATAG CATCAATAGA

                                                     ddeI
                                                 sau3AI      nlaIV
             pleI                                mboI/ndeII[dam-]
             hinfI                               dpnI[dam+]  hgiCI          tru9I
         eam1105I               fokI             dpnII[dam-] banI mnlI      mseI          maeIII
6101 ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA
     TGTGCTGCCC CTCAGTCCGT TGATACCTAC TTGCTTTATC TGTCTAGCGA CTCTATCCAC GGAGTGACTA ATTCGTAACC ATTGACAGTC TGGTTCAAAT
```

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FIG. 3Q

```
                                                    rmaI        sau3AI
                                                 sau3AI hphI    mboI/ndeII[dam-]
                                                 mboI/ndeII[dam-]
                                                 dpnI[dam+]     dpnI[dam+]
                                                 dpnII[dam-]    dpnII[dam-]
                    tru9I                 tru9I bstYI/xhoII     alwI[dam-]         nlaIII          maeII
                    mseI            tru9I mseI  alwI[dam-]    bstYI/xhoII         rcaI          tru9I
                  ahaIII/draI       mseI ahaIII/draI maeI   mboII[dam-]          bspHI         mseI
6201 CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT
     GAGTATATAT GAAATCTAAC TAAATTTTGA AGTAAAAATT AAATTTTCCT AGATCCACTT CTAGGAAAAA CTATTAGAGT ACTGGTTTTA GGGAATTGCA

                                                sau3AI
                                                mboI/ndeII[dam-]
                                                dpnI[dam+]  sau3AI
                                                dpnII[dam-] mboI/ndeII[dam-] thaI
                                               bstYI/xhoII  dpnI[dam+]       fnuDII/mvnI
                                        sau3AI alwI[dam-]   dpnII[dam-]      bstUI
                                        mboI/ndeII[dam-]    alwI[dam-]       bsh1236I
                         hgaI           dpnI[dam+] mboII[dam-]              hinPI       fnu4HI
                      ddeI              dpnII[dam-]        bstYI/xhoII      hhaI/cfoI   bbvI
6301 GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA
     CTCAAAAGCA AGGTGACTCG CAGTCTGGGG CATCTTTTCT AGTTTCCTAG AAGAACTCTA GGAAAAAAAG ACGCGCATTA GACGACGAAC GTTTGTTTTT

                                    sau3AI
                                    mboI/ndeII[dam-]
                                    dpnI[dam+]
                                    dpnII[dam-]
                                   alwI[dam-]
                     aciI         mspI                                bsrI              hinPI
             aciI   nspBII        hpaII       aluI                 maeIII    eco57I     hhaI/cfoI
6401 AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT
     TTGGTGGCGA TGGTCGCCAC CAAACAAACG GCCTAGTTCT CGATGGTTGA GAAAAAGGCT TCCATTGACC GAAGTCGTCT CGCGTCTATG GTTTATGACA

                                                                                                         fnu4HI
                                                                                                  alwNI  bbvI
                                                                                                 bsrI  fnu4HI
        rmaI               haeIII/palI                                                       maeIII     bbvI    bsrI
        maeI      bslI     haeI               scfI     aciI          mnlI
6501 CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT
     GGAAGATCAC ATCGGCATCA ATCCGGTGGT GAAGTTCTTG AGACATCGTG GCGGATGTAT GGAGCGAGAC GATTAGGACA ATGGTCACCG ACGACGGTCA
```

BNSDOCID <WO___9604391A1_I_>

FIG. 3R

```
                                scrFI                                                    aciI                                  hgiAI/aspHI
                                nciI                                                   nspBII                                 bsp1286
                                mspI                                  mspI             fnu4HI                                 bsiHKAI
                                hpaII                                 hpaII            bbvI                                   bmyI
                                dsaV      pleI                      bsaWI            hinPI  mcrI                            apaLI/snoI
                                cauII     hinfI                  maeIII              hhaI/cfoI                              alw44I/snoI   aluI
6601 GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT
     CCGCTATTCA GCACAGAATG GCCCAACCTG AGTTCTGCTA TCAATGGCCT ATTCCGCGTC GCCAGCCCGA CTTGCCCCCC AAGCACGTGT GTCGGGTCGA

                                                                                                                          mspI
                                                                                hinPI                                     hpaII
                                                                                hhaI/cfoI                                 bslI   fnu4HI
                                        ddeI      scfI                        haeII                              aciI     bsaWI  aciI
6701 TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG
     ACCTCGCTTG CTGGATGTGG CTTGACTCTA TGGATGTCGC ACTCGTAACT CTTTCGCGGT GCGAAGGGCT TCCCTCTTTC CGCCTGTCCA TAGGCCATTC

                                                scrFI
                                                mvaI            scrFI
                                                ecoRII    mvaI
                                                dsaV      ecoRII
                                                bstNI             dsaV
                                hinPI mnlI      bsaJI             bstNI                                                        taqI
                                hhaI/cfoI   aluI apyI[dcm+]       apyI[dcm+]                                 mnlI drdI    hgaI
6801 CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT
     GCCGTCCCAG CCTTGTCCTC TCGCGTGCTC CCTCGAAGGT CCCCCTTTGC GGACCATAGA AATATCAGGA CAGCCCAAAG CGGTGGAGAC TGAACTCGCA

                                                                                                    haeIII/palI
                                                                haeIII/palI       scrFI
                                                               fnu4HI             mvaI bslI
                                                               aciI               ecoRII
                                                               thaI bslI          dsaV                          nlaIII
                                                               fnuDII/mvnI        bstNI                              nspI
                                        nlaIV                  bstUI              apyI[dcm+]     haeIII/palI     nspHI
                sfaNI                aciI                      bsh1236I       nlaIV  haeI        haeI           aflIII
6901 CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA
     GCTAAAAACA CTACGAGCAG TCCCCCCGCC TCGGATACCT TTTTGCGGTC GTTGCGCCGG AAAAATGCCA AGGACCGGAA AACGACCGGA AAACGAGTGT

                                                                                    fnu4HI                fnu4HI
                                                                                    bbvI                  bbvI pleI
                                tfiI                                                                      hinPI  hinfI
                                hinfI                        aciI          aluI     aciI   fnu4HI    mcrI   hhaI/cfoI
7001 TGTTCTTTCC TGCGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC TCGCCGCAGC CGAACGACCG AGCGCAGCGA
     ACAAGAAAGG ACGCAATAGG GGACTAAGAC ACCTATTGGC ATAATGGCGG AAACTCACTC GACTATGGCG AGCGGCGTCG GCTTGCTGGC TCGCGTCGCT
```

BNSDOCID <WO__9604391A1_1_>

FIG. 3S

```
                                                                                                                    thaI
                                                                                                                    fnuDII/mvnI
                                                                                                                    bstUI
                                                                                                                    bsh1236I
                                                                                                                   hinPI
                                                                                                                    hhaI/cfoI
                                                                                                                  thaI
                                                                                                                  fnuDII/mvnI
                                  sapI  hinPI                                                                     bstUI   haeIII/palI        aluI
                                  mboII hhaI/cfoI                                                                 bsh1236I           tru9I  pvuII
                                  earI/ksp632I                                 mnlI     bslI    eaeI  tfiI aseI/asnI/vspI
                        mnlI  aciI    haeII                                  aciI       aciI    cfrI  hinfI mseI   nspBII                    bsrI
7101 GTCAGTGAGC GAGGAAGCGG AAGAGCGCCC AATACGCAAA CCGCCTCTCC CCGCGCGTTG GCCGATTCAT TAATCCAGCT GGCACGACAG GTTTCCCGAC
     CAGTCACTCG CTCCTTCGCC TTCTCGCGGG TTATGCGTTT GGCGGAGAGG GGCGCGCAAC CGGCTAAGTA ATTAGGTCGA CCGTGCTGTC CAAAGGGCTG

                                                                                                                    scrFI
                                                                                                                    mvaI
                                                                                                                    ecoRII
                                                                                                                    dsaV
                                          tru9I                                                                   nlaIV bstNI
                                 hinPI    mseI      maeIII                                                        hgiCI apyI[dcm+]                mspI
                    aciI         hhaI/cfoI aseI/asnI/vspI  mnlI                                                   banI bsaJI                      hpaII
7201 TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT ACCTCACTCA TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG
     ACCTTTCGCC CGTCACTCGC GTTGCGTTAA TTACACTCAA TGGAGTGAGT AATCCGTGGG GTCCGAAATG TGAAATACGA AGGCCGAGCA TACAACACAC

                                                                                                                      tru9I
                                                                                                                      mseI
                                                                                                                    aseI/asnI/vspI
                      aciI                                                                                          xmnI
                    bsrBI                                                 aluI      nlaIII                        asp700
7301 GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT TACGAATTAA
     CTTAACACTC GCCTATTGTT AAAGTGTGTC CTTTGTCGAT ACTGGTACTA ATGCTTAATT
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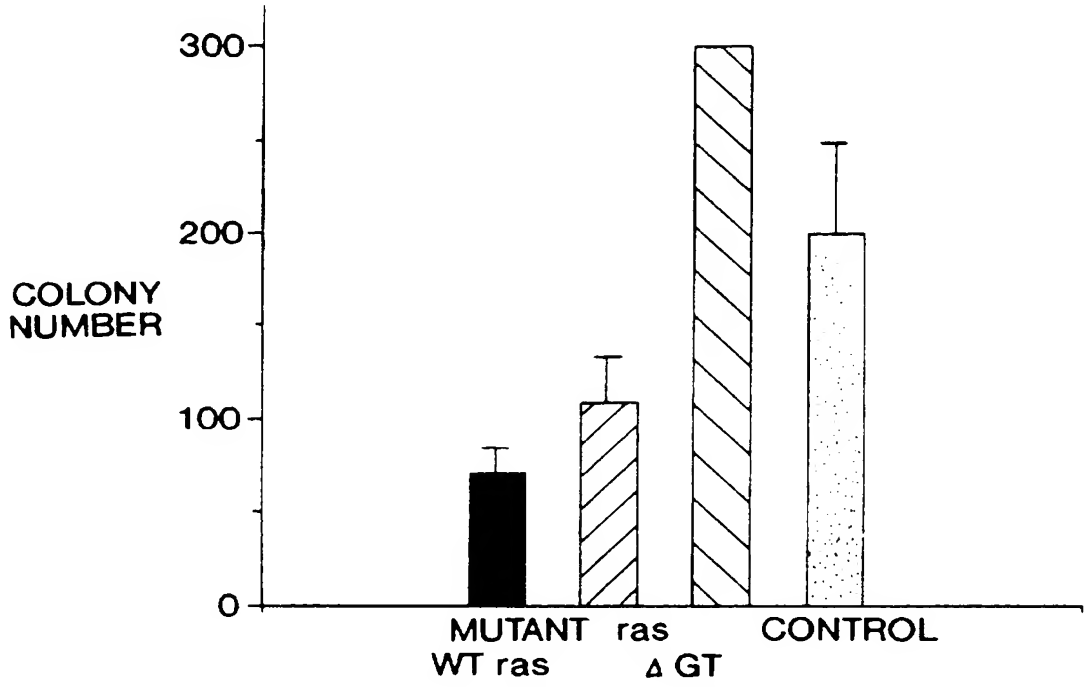

FIG. 4

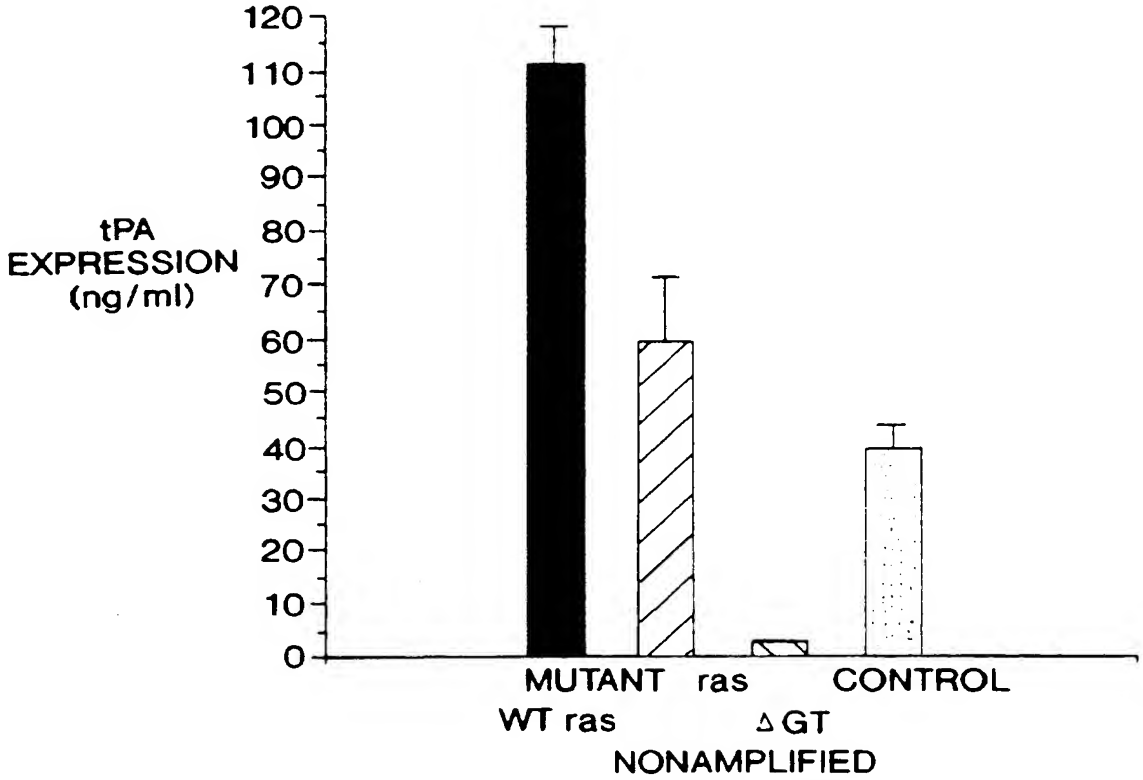

FIG. 5A

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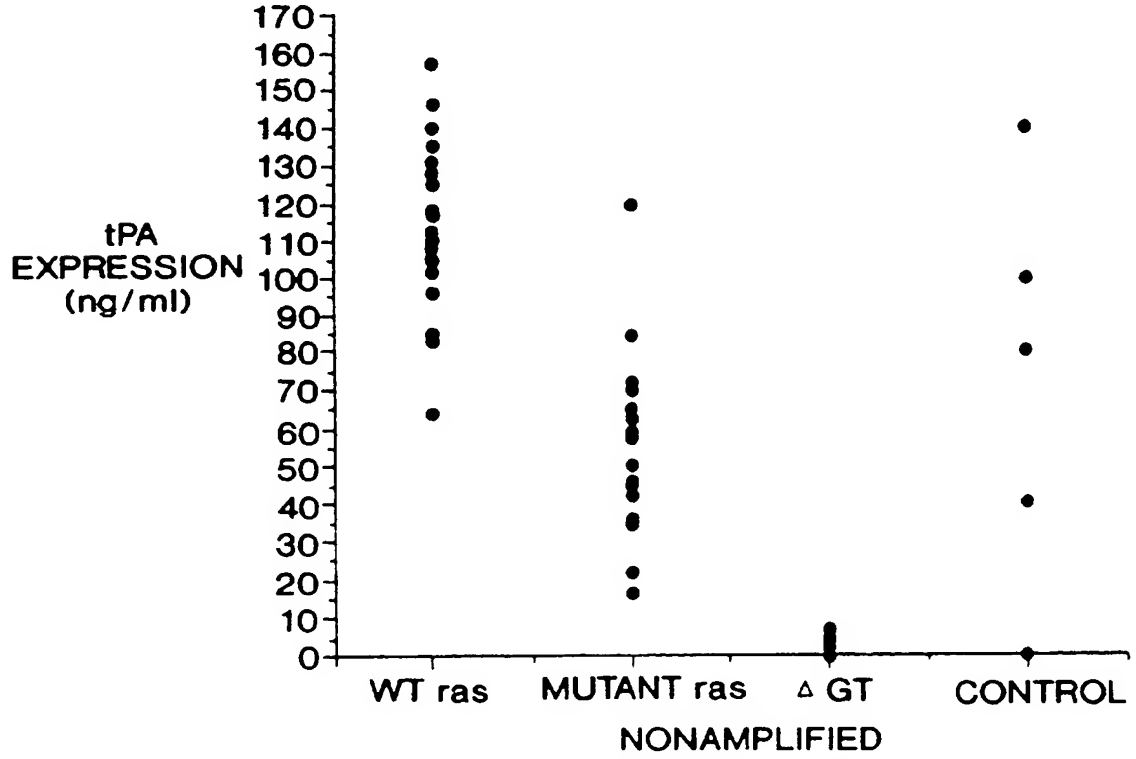
tPA
EXPRESSION
(ng/ml)
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
WT ras
MUTANT ras
Δ GT
CONTROL
NONAMPLIFIED

FIG. 5B

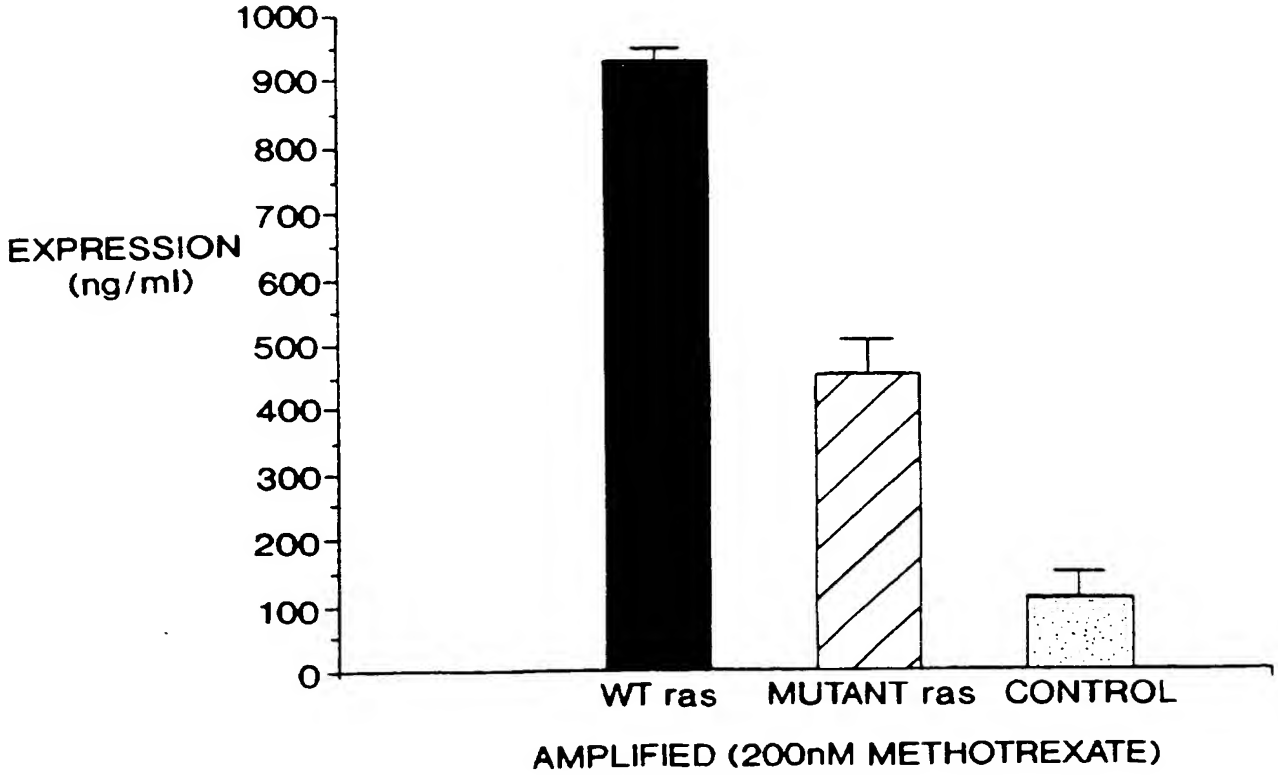
EXPRESSION
(ng/ml)
1000
900
800
700
600
500
400
300
200
100
0
WT ras
MUTANT ras
CONTROL
AMPLIFIED (200nM METHOTREXATE)

FIG. 5C

BNSDOCID <WO__9604391A1_1_>

FIG. 6A

```
      aluI
     sstI
     sacI
     hgiJII
     hgiAI/aspHI
     ecl136II
     bsp1286                                                                    thaI
     bsiHKAI                                                                    fnuDII/mvnI
     bmyI                      rmaI    tru9I                                    bstUI
     banII                     maeI    mseI                                     bsh1236I
   taqI                       speI    aseI/asnI/vspI                  bslI      aciI maeIII
 1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
   AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                 scrFI
                 mvaI
                 ecoRII
                 dsaV
               aciI
              bglI bstNI                          maeII
             sau96I                              hinlI/acyI
             haeIII/palI     aciI                ahaII/bsaHI
             asuI  apyI[dcm+]                aciI     aatII          maeII          maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

     maeII                                                                      maeII
    hinlI/acyI                                                                  hinlI/acyI
    ahaII/bsaHI                                    rsaI                 rsaI    ahaII/bsaHI
    aatII                              bglI        csp6I          ndeI  csp6I   aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

           scrFI
           mvaI
           ecoRII
        aciI                                                                    nlaIII
       bglI  dsaV                                                          styI
      sau96I bstNI           rsaI                                  maeII      ncoI
      haeIII/palI            csp6I                          rsaI   snaBI      dsaI hphI  aciI
      asuI   apyI[dcm+]   bsrI  nlaIII                      csp6I  bsaAI      bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 6B

```
                                                                                                                maeII
                                                                                                               hinlI/acyI                nlaIV
                                rsaI                                  pleI                                     ahaII/bsaHI               hgiCI
                                csp6I                          aciI   hinfI                    bsmAI            aatII                     banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                                                    aluI
                                                                                                                                    sstI
                                                                                                                                    sacI
                                                                                                                                    hgiJII
                                                                                                                                    hgiAI/aspHI
                                                                                                                                    ecl136II
                                                                                                                                    bsp1286
                                                                                                                                    bsiHKAI
                                                                                                                rsaI                bmyI
                                        maeIII     aciI        hgaI         aciI          csp6I     mnlI                            banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                                                 haeIII/palI
                                                                                                                mcrI
                                                                                                                eagI/xmaIII/eclXI
                                                                                                                eaeI
                                                                                                                cfrI
                                                                                                               fnu4HI
                                                                                                               aciI
                                                                                                              thaI
                                                                                                              fnuDII/mvnI
                                    esp3I
                                scrFI                                                          sau96I          sacII/sstII
                                mvaI bsmAI                                                          avaII      nspBII
                                ecoRII                                                         asuI            kspI  scrFI
                                dsaV                                                          nlaIV            dsaI  nciI
                                bstNI  hinlI/acyI                                                 scrFI         bglI bslI  mspI
                                apyI[dcm+]                                                        nciI     sau3AI mnlI bstUI
                           sau3AI gsuI/bpmI                                                       mspI     mboI/ndeII[dam-] hpaII
                           mboI/ndeII[dam-]                                              mboII    hpaII    dpnI[dam+] bsaJI dsaV
                           dpnI[dam+]  hgaI  fokI                                        bpuAI    dsaV     dpnII[dam-] bsh1236I
                           dpnII[dam-] ahaII/bsaHI                        mnlI           bbsI     cauII    alwI[dam-] aciI  cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

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FIG. 6C

```
                tfiI
              aciI
             thaI hinfI                                                                    fnu4HI
             fnuDII/mvnI                          aciI                                     bbvI
             bstUI                              rsaI                                    nspBII          taqI
             bsh1236I                           csp6I   scfI          mnlI              aciI       nlaIII
701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT
    AACCTTGCGC CTAAGGGGCA CGGTTCTCAC GACATTCATG GCGGATATCT CGCTATTCTC CTAAAATAGG GGCGACGGTA GTACCAAGCT GGTAACTTGA

                                                                                          thaI
                                                                                          fnuDII/mvnI
                                                                                          bstUI
                                                                                          bsh1236I
                                                                                        mluI
                                                                          bsrBI         aflIII      rsaI
                      pflMI                             bsmAI           aciI      xmnI            csp6I
    sfaNI             bslI                              bsaI          mnlI   ddeI asp700        scaI
801 GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC
    CGTAGCAGCG GCACAGGGTT TTATACCCCT AACCGTTCTT GCCTCTGGAT GGGACGGGAG GCGAGTCCTT GCGCAAGTTC ATGAAGGTTT CTTACTGGTG

                                                        scrFI
                                                        mvaI
                                                        ecoRII
                 eco57I                                 dsaV
                mboII                   tfiI            bstNI                          tfiI        tru9I          tru9I
               earI/ksp632I             hinfI           apyI[dcm+]                mboII taqI     mseI           mseI
             mnlI                     alwNI    hphI                 sexAI              ddeI    hinfI     ahaIII/draI   aseI/asnI/vspI
901 AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT
    TTGGAGAAGT CACCTTCCAT TTGTCTTAGA CCACTAATAC CCATCCTTTT GGACCAAGAG GTAAGGACTC TTCTTAGCTG GAAATTTCCT GTCTTAATTA

                                          aluI
                                          sstI
                                          sacI
                                          hgiJII
                                          hgiAI/aspHI
                                          ecl136II
                                          bsp1286
                                          bsiHKAI
                                          bmyI                                        tru9I              mspI
                                          banII                                 sfaNI mseI               hpaII
                ddeI                   bslI    mnlI                 bstXI      fokI      aflII/bfrI         bsaWI
1001 ATAGTTCTCA GTAGAGAACT CAAAGAACCA CCACGAGGAG CTCATTTTCT TGCCAAAAGT TTGGATGATG CCTTAAGACT TATTGAACAA CCGGAATTGG
     TATCAAGAGT CATCTCTTGA GTTTCTTGGT GGTGCTCCTC GAGTAAAAGA ACGGTTTTCA AACCTACTAC GGAATTCTGA ATAACTTGTT GGCCTTAACC
```

FIG. 6D

```
                                                                             haeIII/palI
                                                                           haeI
                                               scrFI          scrFI                                  nlaIII
                                               mvaI                 mvaI                                      sau3AI
                                               ecoRII         ecoRII                              mboI/ndeII[dam-]
                                               dsaV    tfiI   dsaV  pleI                          dpnI[dam+]
                                               bstNI      nlaIII    bstNI        ddeI                     dpnII[dam-]
          accI  nlaIII           mnlI          apyI[dcm+]  hinfI    apyI[dcm+]     hinfI     maeIII   alwI[dam-]
1101 CAAGTAAAGT AGACATGGTT TGGATAGTCG GAGGCAGTTC TGTTTACCAG GAAGCCATGA ATCAACCAGG CCACCTTAGA CTCTTTGTGA CAAGGATCAT
     GTTCATTTCA TCTGTACCAA ACCTATCAGC CTCCGTCAAG ACAAATGGTC CTTCGGTACT TAGTTGGTCC GGTGGAATCT GAGAAACACT GTTCCTAGTA

                                                                                   mnlI
                                                                              hinlI/acyI           scrFI
                                                                              ahaII/bsaHI          mvaI
                                                                           scrFI          ecoRII
                                                                           mvaI    ecoNI           dsaV
                                                                           ecoRII     sau96I
                                                                           dsaV       avaII
                maeII                                                      bstNI   bslI     asuI    mnlI
              aflIII                                                       apyI[dcm+]      mnlI  bstNI
      apoI     maeIII                                   mnlI            bsaJI hgaI     ddeI     apyI[dcm+]
1201 GCAGGAATTT GAAAGTGACA CGTTTTTCCC AGAAATTGAT TTGGGGAAAT ATAAACCTCT CCCAGAATAC CCAGGCGTCC TCTCTGAGGT CCAGGAGGAA
     CGTCCTTAAA CTTTCACTGT GCAAAAAGGG TCTTTAACTA AACCCCTTTA TATTTGGAGA GGGTCTTATG GGTCCGCAGG AGAGACTCCA GGTCCTCCTT

                                                                                            ppu10I
                                                          sfaNI                             nsiI/avaIII
      sfaNI                  accI     mboII          mboII                        mnlI    aluI
1301 AAAGGCATCA AGTATAAGTT TGAAGTCTAC GAGAAGAAAG ACTAACAGGA AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA
     TTTCCGTAGT TCATATTCAA ACTTCAGATG CTCTTCTTTC TGATTGTCCT TCTACGAAAG TTCAAGAGAC GAGGGGAGGA TTTCGATACG TAAAAATATT

                                                     fnu4HI
      nlaIII                                         aciI
     styI                                           thaI
     ncoI                                           fnuDII/mvnI tru9I
     dsaI                            styI           bstUI      mseI                                      maeIII
     bsaJI                           bsaJI          bsh1236I  aseI/asnI/vspI                            hphI
1401 GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA
     CTGGTACCCT GAAAACGACC GAAATCTGGG GGAACCGAAG CAATCTTGCG CCGATGTTAA TTATGTATTG GAATACATAG TATGTGTATC TAAATCCACT
```


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FIG. 6E

```
                                                      scrFI
                                                      mvaI
                                                      ecoRII
                                                      dsaV
                                                      bstNI
                                                      apyI[dcm+]      mnlI
                                                    gsuI/bpmI         bsaJI
                                                maeIII      hincII/hindII
     scfI       fokI
1501 CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA GGTCAACTGC ACCTCGGTTC
     GTGATATCTT ATTGTAGGTG AAACGGAAAG AGAGGTGTCC ACAGTGAGGT CCAGTTGACG TGGAGCCAAG

                 haeIII/palI
                 eaeI
          cfrI
               mspI
         hpaII
        scrFI
               nciI
      ecoRI    dsaV
 taqI apoI     cauII
claI/bsp106  bsaJI      aluI
TATCGATTGA ATTCCCCGGC CATAGCTGTC
ATAGCTAACT TAAGGGGCCG GTATCGACAG

                                                        gsuI/bpmI
                                                        scrFI
                                                        mvaI
                                                        ecoRII
                                                        dsaV
                                          nspBII        bstNI
       mnlI                          fnu4HI       hgiAI/aspHI
      haeIII/palI                 fnu4HI aciI     bsp1286
      sau96I                      bbvI  fnu4HI    bsiHKAI     aluI
      asuI                                        bmyI apyI[dcm+]
    nlaIII                   bspMI   bbvI
1601 TGGCATGGGC CTCTCCACCG TGCCTGACCT GCTGCTGCCG CTGGTGCTCC TGGAGCTGTT GGTGGGAATA
     ACCGTACCCG GAGAGGTGGC ACGGACTGGA CGACGACGGC GACCACGAGG ACCTCGACAA CCACCCTTAT

                                mnlI
                                ecoNI
   ddeI                           sau96I
  mnlI                            nlaIV
  eco81I                          avaII
  bsu36I/mstII/sauI               asuI  hphI
bslI                            bsrI  bslI
TACCCCTCAG GGGTTATTGG ACTGGTCCCT
ATGGGGAGTC CCCAATAACC TGACCAGGGA

      rmaI
      maeI
     styI
     bsaJI                                   styI
     blnI          mboII                     bsaJI
     avrII         earI/ksp632I                              mnlI
1701 CACCTAGGGG ACAGGGAGAA GAGAGATAGT GTGTGTCCCC AAGGAAAATA TATCCACCCT CAAAATAATT
     GTGGATCCCC TGTCCCTCTT CTCTCTATCA CACACAGGGG TTCCTTTTAT ATAGGTGGGA GTTTTATTAA

             rsaI
 taqI        csp6I           nlaIV
CGATTTGCTG TACCAAGTGC CACAAAGGAA
GCTAAACGAC ATGGTTCACG GTGTTTCCTT
```


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FIG. 6F

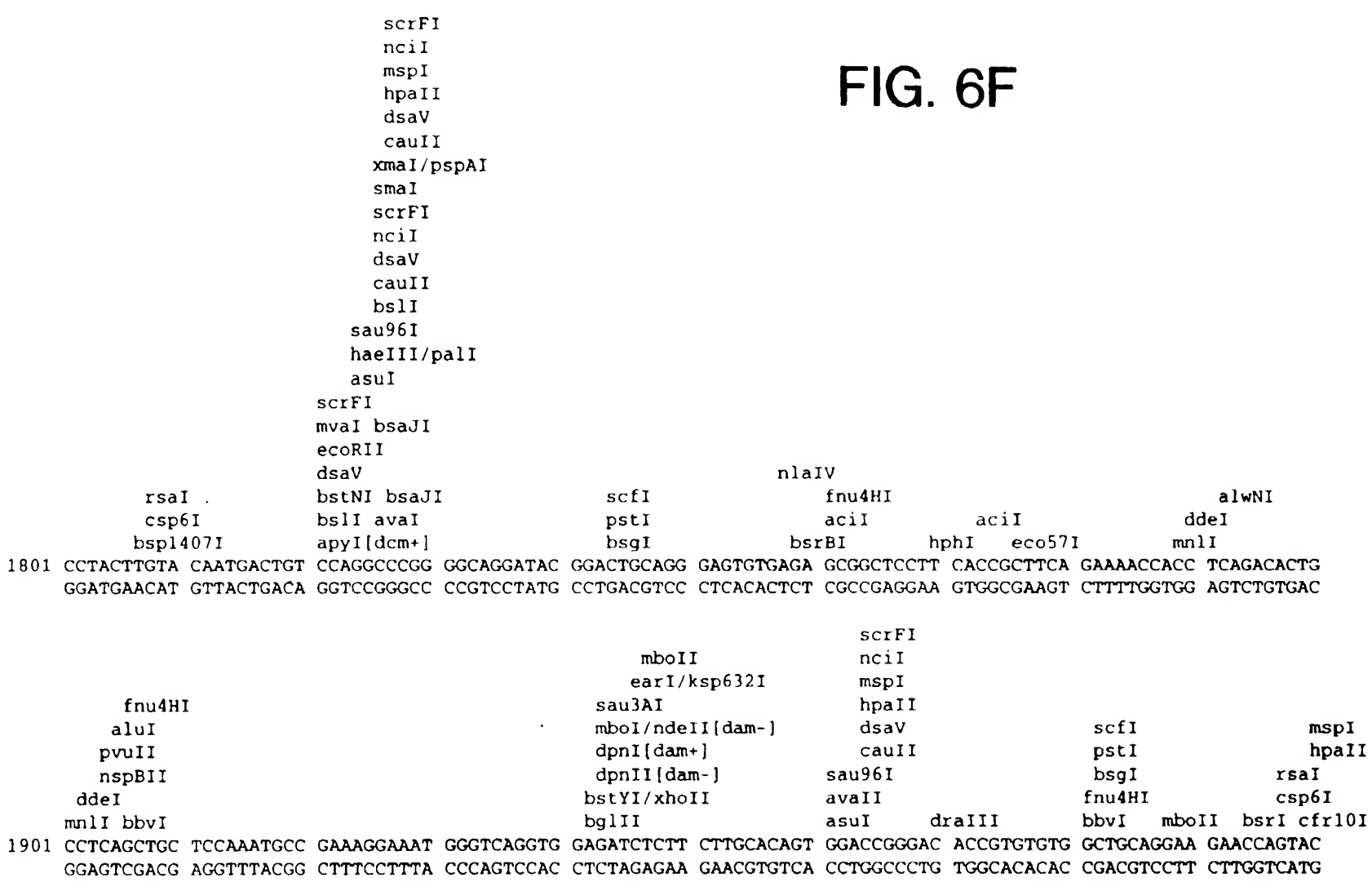

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FIG. 6G

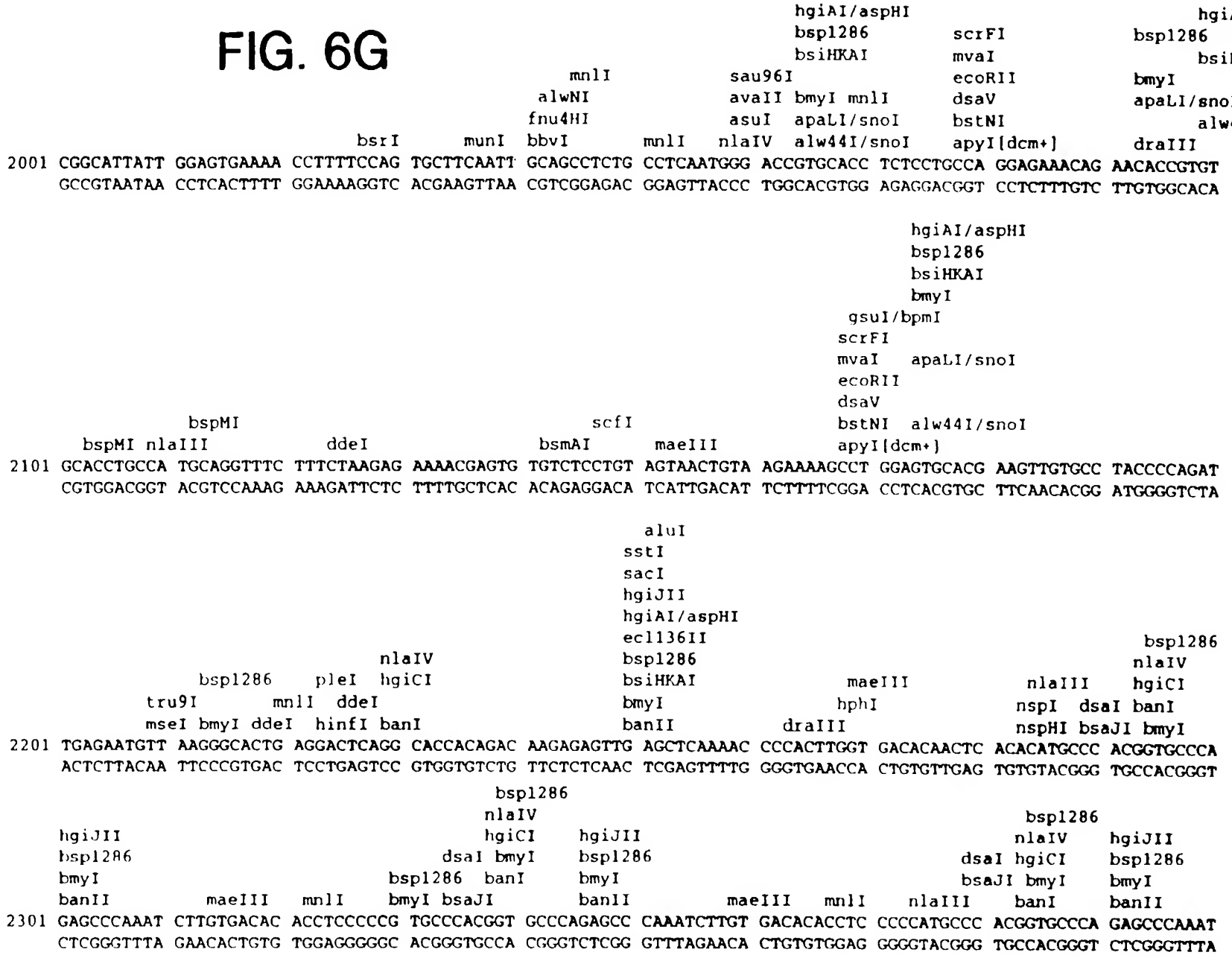

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FIG. 6H

```
                                                                eam1105I
                                                               sau96I
                                                       scrFI
                                   bsp1286             mvaI    avaII
                                  nlaIV                ecoRII
                                  hgiCI                dsaV
                              dsaI bmyI                bstNI   asuI      mboII mboII
                              bsaJI                    bsaJI mnlI       bpuAI earI/ksp632I      styI
        maeIII   mnlI   nlaIII    banI   alwNI         apyI[dcm+]       bbsI mnlI               bsaJI
2401 CTTGTGACAC ACCTCCCCCA TGCCCACGGT GCCCAGCACC TGAACTCCTG GGAGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG ATACCCTTAT
     GAACACTGTG TGGAGGGGGT ACGGGTGCCA CGGGTCGTGG ACTTGAGGAC CCTCCTGGCA GTCAGAAGGA GAAGGGGGGT TTTGGGTTCC TATGGGAATA

             sau96I
             nlaIV
             avaII
             asuI           maeII
           mspI            pmlI
           hpaII           eco72I                                        sau96I
          scrFI       mnlI bsaAI                              drdI       avaII             maeII
          nciI       ddeI maeIII                          mboII     mnlI                  bsaAI
          dsaV      eco81I bbrPI                          bpuAI   bsaJI   bsrI           rsaI
          cauII     bsu36I/mstII/sauI          maeII      bbsI   avaI    asuI            csp6I             mnlI
2501 GATTTCCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC GTGAGCCACG AAGACCCCGA GGTCCAGTTC AAGTGGTACG TGGACGGCGT GGAGGTGCAT
     CTAAAGGGCC TGGGGACTCC AGTGCACGCA CCACCACCTG CACTCGGTGC TTCTGGGGCT CCAGGTCAAG TTCACCATGC ACCTGCCGCA CCTCCACGTA

                       aciI
                      thaI
                      fnuDII/mvnI
                      bstUI
                      bsh1236I
                     sacII/sstII
                     nspBII                                                             scrFI
                     kspI                                                               mvaI bsrI
                     dsaI                                                               ecoRII
                     bsaJI                                                              dsaV
                     aciI                                               mnlI      ecoNI bstNI                     rsaI
                    fnu4HI  mnlI                  maeII             hgaI   hphI   bslI  apyI[dcm+]                csp6I
2601 AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TTCAACAGCA CGTTCCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAC GGCAAGGAGT
     TTACGGTTCT GTTTCGGCGC CCTCCTCGTC AAGTTGTCGT GCAAGGCACA CCAGTCGCAG GAGTGGCAGG ACGTGGTCCT GACCGACTTG CCGTTCCTCA
```


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BNSDOCID <WO 9604391A1_I_>

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FIG. 6I

```
                bsmAI                                                                                                  rsaI
                bsaI              mnlI              taqI                                        avaI                 csp6I
                                                                                                                     bsp1407I     bslI
2701 ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAA CCAAAGGACA GCCCCGAGAA CCACAGGTGT ACACCCTGCC
     TGTTCACGTT CCAGAGGTTG TTTCGGGAGG GTCGGGGGTA GCTCTTTTGG TAGAGGTTTT GGTTTCCTGT CGGGGCTCTT GGTGTCCACA TGTGGGACGG

          scrFI
          nciI
          mspI
          hpaII
          dsaV
          cauII
          xmaI/pspAI
          smaI
          scrFI                    scrFI          scrFI
          nciI                     mvaI                mvaI
          dsaV                     ecoRII         ecoRII
          cauII                    dsaV           dsaV                                                    aciI
          bsaJI                    bstNI                bstNI                                  dsaI              nspBII
        fokI                       apyI[dcm+]           apyI[dcm+]                             bslI              fnu4HI fnu4HI
     bslI avaI  mnlI               sexAI           bspMI                                     bsaJI             bbvI   bbvI
2801 CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA GAGCAGCGGG
     GGGTAGGGCC CTCCTCTACT GGTTCTTGGT CCAGTCGGAC TGGACGGACC AGTTTCCGAA GATGGGGTCG CTGTAGCGGC ACCTCACCCT CTCGTCGCCC

                                                                                                  dsaI
          mspI                                    pleI                    mnlI                hphI                    fnu4HI
          hpaII                         mnlI  nlaIII hinfI     nlaIV   mboII   scfI      aluI  bsaJI         bspMI   bbvI
2901 CAGCCGGAGA ACAACTACAA CACCACGCCT CCCATGCTGG ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC
     GTCGGCCTCT TGTTGATGTT GTGGTGCGGA GGGTACGACC TGAGGCTGCC GAGGAAGAAG GAGATGTCGT TCGAGTGGCA CCTGTTCTCG TCCACCGTCG

                                                                                                                    scrFI
                                                                                                                    nciI
                                                                                                                    mspI
                                                                                                                    hpaII
                                                                                                                    dsaV
                                                                                                  scrFI              cauII
                                                                                                  nciI               bsaJI
                                                                                                  mspI              haeIII/palI
                                                                                                  hpaII             mcrI
                                                                                                  dsaV              eagI/xmaIII/eclXI
          xmnI mboII                                                                  sapI        mboII  mnlI      bsmAI  eaeI
          asp700      nlaIII     sfaNI   mnlI          aciI                         earI/ksp632I  bslI   cauII          cfrI
3001 AGGGGAACAT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCGC TTCACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAATGAG TGCGACGGCC
     TCCCCTTGTA GAAGAGTACG AGGCACTACG TACTCCGAGA CGTGTTGGCG AAGTGCGTCT TCTCGGAGAG GGACAGAGGC CCATTTACTC ACGCTGCCGG
```

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FIG. 6J

```
                rmaI
              mnlI
            sau3AI
            mboI/ndeII[dam-]                    styI
            dpnI[dam+]                        aciI
            dpnII[dam-]  taqI                fnu4HI   sau96I
            alwI[dam-]  salI   scfI          sfiI ncoI haeIII/palI
          nlaIV  maeI  hincII/hindII         haeIII/palI
          bstYI/xhoII  accI   pstI         eaeI  dsaI asuI                aluI
          bamHI xbaI pleI     bsgI   aluI cfrI  bsaJI                fnu4HI
          alwI[dam-] hinfI bspMI    hindIII bglI nlaIII                bbvI          maeIII             sfaNI   apoI
3101 GGGGATCCTC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT AAAGCAATAG CATCACAAAT
     CCCCTAGGAG ATCTCAGCTG GACGTCTTCG AACCGGCGGT ACCGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA TTTCGTTATC GTAGTGTTTA

                                                                                                  sau3AI
                                                                                                  mboI/ndeII[dam-]
                                                                                                  dpnI[dam+]
                                                                                                  dpnII[dam-]
                                                                                                 pvuI/bspCI
                                                                                                 mcrI
                                                                                               taqI[dam-]   tru9I
                                                                                              claI/bsp106[dam-]
                                                                                            sau3AI         mseI
                                                                                            mboI/ndeII[dam-]
                                                                                            dpnI[dam+] xmnI        hinPI
                                                                                            dpnII[dam-]  aseI/asnI/vspI
                                                                                     nlaIII  alwI[dam-]  asp700      hhaI/cfoI
3201 TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT CGATCGGGAA TTAATTCGGC
     AAGTGTTTAT TTCGTAAAAA AAGTGACGTA AGATCAACAC CAAACAGGTT TGAGTAGTTA CATAGAATAG TACAGACCTA GCTAGCCCTT AATTAAGCCG

                                                 rsaI
                                                 csp6I
                haeI                            nlaIV
              styI                              kpnI
              ncoI                              hgiCI
              dsaI haeIII/palI                  banI                          aluI
        fnu4HI nlaIII                           asp718     mnlI           pvuII
        bbvI  bsaJI             mnlI      mnlI  acc65I   ddeI aciI        nspBII
3301 GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC AGCTGTGGAA TGTGTGTCAG TTAGGGTGTG
     CGTCGTGGTA CCGGACTTTA TTGGAGACTT TCTCCTTGAA CCAATCCATG GAAGACTCCG CCTTTCTTGG TCGACACCTT ACACACAGTC AATCCCACAC
```

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FIG. 6K

```
                nlaIV                                                                                                  nlaIV
              scrFI                                                                  scrFI        scrFI
              mvaI                                  ppu10I                             mvaI                 mvaI
              ecoRII                              nsiI/avaIII                        ecoRII       ecoRII
              dsaV                               nlaIII                              dsaV         dsaV
              bstNI                                 sphI                                bstNI                bstNI
              apyI{dcm+}                            nspI sfaNI                          apyI{dcm+}           apyI{dcm+}
             bsaJI                                  nspHI                             sexAI              bsaJI
3401 GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC CCAGGCTCCC CAGCAGGCAG
     CTTTCAGGGG TCCGAGGGGT CGTCCGTCTT CATACGTTTC GTACGTAGAG TTAATCAGTC GTTGGTCCAC ACCTTTCAGG GGTCCGAGGG GTCGTCCGTC

                  sfaNI
                 ppu10I
                 nsiI/avaIII
                nlaIII
               sphI
               nspI                                                                                  aciI                                                                  bslI
               nspHI                                                  aciI          aciI  fokI                 aciI bsrI  aciI                                        aciI
3501 AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC CCAGTTCCGC CCATTCTCCG
     TTCATACGTT TCGTACGTAG AGTTAATCAG TCGTTGGTAT CAGGGCGGGG ATTGAGGCGG GTAGGGCGGG GATTGAGGCG GGTCAAGGCG GGTAAGAGGC

                                                                                                                                                                    rmaI
                                                                                                                                                                   styI
                                                                                                                                                                   bsaJI
                                                                                                                                                                   blnI
                                                      fnu4HI                                                                                                       avrII
       nlaIII                                         sfiI mnlI                                                                                                   haeIII/palI
      styI                                            haeIII/palI                                                                                                 stuI
      ncoI                                      bsaJI bglI          ddeI                                                                                        haeI
      dsaI                                     haeIII/palI bsaJI  mnlI  aluI                                  mnlI                                                mnlI maeI
      bsaJI                                   mnlI  mnlI aciI    haeIII/palI                                mnlI
3601 CCCCATGGCT GACTAATTTT TTTTATTTAT GCAGAGGCCG AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG AGGCTTTTTT GGAGGCCTAG
     GGGGTACCGA CTGATTAAAA AAAATAAATA CGTCTCCGGC TCCGGCGGAG CCGGAGACTC GATAAGGTCT TCATCACTCC TCCGAAAAAA CCTCCGGATC

                                                                                                  scrFI
                                                                                                  mvaI
                                                                                                  ecoRII
                                                                                                  dsaV
                      tru9I                haeIII/palI                                            bstNI
                     hpaI                 eaeI                                                    apyI{dcm+}                tru9I          fnu4HI
                     hincII/hindII        cfrI                                  bsrI              bsaJI   maeIII           mseI           bbvI
                  aluI mseI  aluI      bsrI                              maeII maeIII
3701 GCTTTTGCAA AAAGCTGTTA ACAGCTTGGC ACTGGCCGTC GTTTTACAAC GTCGTGACTG GGAAAACCCT GGCGTTACCC AACTTAATCG CCTTGCAGCA
     CGAAAACGTT TTTCGACAAT TGTCGAACCG TGACCGGCAG CAAAATGTTG CAGCACTGAC CCTTTTGGGA CCGCAATGGG TTGAATTAGC GGAACGTCGT
```


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BNSDOCID <WO 9604391A1 >

FIG. 6L

```
                                                   sau3AI                                                hinPI
                                         sau96I    mboI/ndeII[dam-]                                        hhaI/cfoI
                                         haeIII/palI                                                     nlaIV
                                         asuI      dpnI[dam+]                                            narI
                aluI                    mnlI       dpnII[dam-]                                           kasI
                pvuII                 mboII  aciI pvuI/bspCI                                             hinlI/acyI
      fokI      nspBII                earI/ksp632I mcrI                         bglI                     hgiCI
3801  CATCCCCCCT TCGCCAGCTG GCGTAATAGC GAAGAGGCCC GCACCGATCG CCCTTCCCAA CAGTTGCGTA GCCTGAATGG CGAATGGCGC CTGATGCGGT
      GTAGGGGGGA AGCGGTCGAC CGCATTATCG CTTCTCCGGG CGTGGCTAGC GGGAAGGGTT GTCAACGCAT CGGACTTACC GCTTACCGCG GACTACGCCA
                                                                                                         haeII      aciI
                                                                                                         banI     sfaNI
                                                                                                         ahaII/bsaHI

                                                                                                           aciI
                                                                                                         fnu4HI
                                                                           hinPI                         aciI
                                                                          thaI                          thaI
                                                                          fnuDII/mvnI                   fnuDII/mvnI
                                                                          bstUI scfI     hinPI          bstUI
                                                                          bsh1236I       hhaI/cfoI hinPI
                                                                        rsaI hhaI/cfoI fnu4HI  tru9I bsh1236I
                 sfaNI    aciI        aciI  maeII                       csp6I  bslI    aciI    mseI hhaI/cfoI
3901  ATTTTCTCCT TACGCATCTG TGCGGTATTT CACACCGCAT ACGTCAAAGC AACCATAGTA CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG
      TAAAAGAGGA ATGCGTAGAC ACGCCATAAA GTGTGGCGTA TGCAGTTTCG TTGGTATCAT GCGCGGGACA TCGCCGCGTA ATTCGCGCCG CCCACACCAC

           fnu4HI
         hinPI
         hhaI/cfoI                         hinPI
        thaI                               hhaI/cfoI
        fnuDII/mvnI                      rmaI                                                       mspI
        bstUI                        hinPI  haeII                                                   hpaII
        bsh1236I     aciI            hhaI/cfoI   bsrBI                                             naeI
      maeIII bbvI  maeIII           haeII maeI   aciI            mboII                    maeII  cfr10I
4001  GTTACGCGCA GCGTGACCGC TACACTTGCC AGCGCCCTAG CGCCCGCTCC TTTCGCTTTC TTCCCTTCCT TTCTCGCCAC GTTCGCCGGC TTTCCCCGTC
      CAATGCGCGT CGCACTGGCG ATGTGAACGG TCGCGGGATC GCGGGCGAGG AAAGCGAAAG AAGGGAAGGA AAGAGCGGTG CAAGCGGCCG AAAGGGGCAG

                nlaIV
                hgiJII
                bsp1286                                 nlaIV                                        maeII    haeIII/palI
                bmyI                                    hgiCI  taqI                                 draIII   sau96I
       aluI     banII        nlaIV                      banI mnlI                        hphI       bsaAI    asuI
4101  AAGCTCTAAA TCGGGGGCTC CCTTTAGGGT TCCGATTTAG TGCTTTACGG CACCTCGACC CCAAAAAACT TGATTTGGGT GATGGTTCAC GTAGTGGGCC
      TTCGAGATTT AGCCCCCGAG GGAAATCCCA AGGCTAAATC ACGAAATGCC GTGGAGCTGG GGTTTTTTGA ACTAAACCCA CTACCAAGTG CATCACCCGG
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BNSDOCID <WO___9604391A1_I_>

FIG. 6M

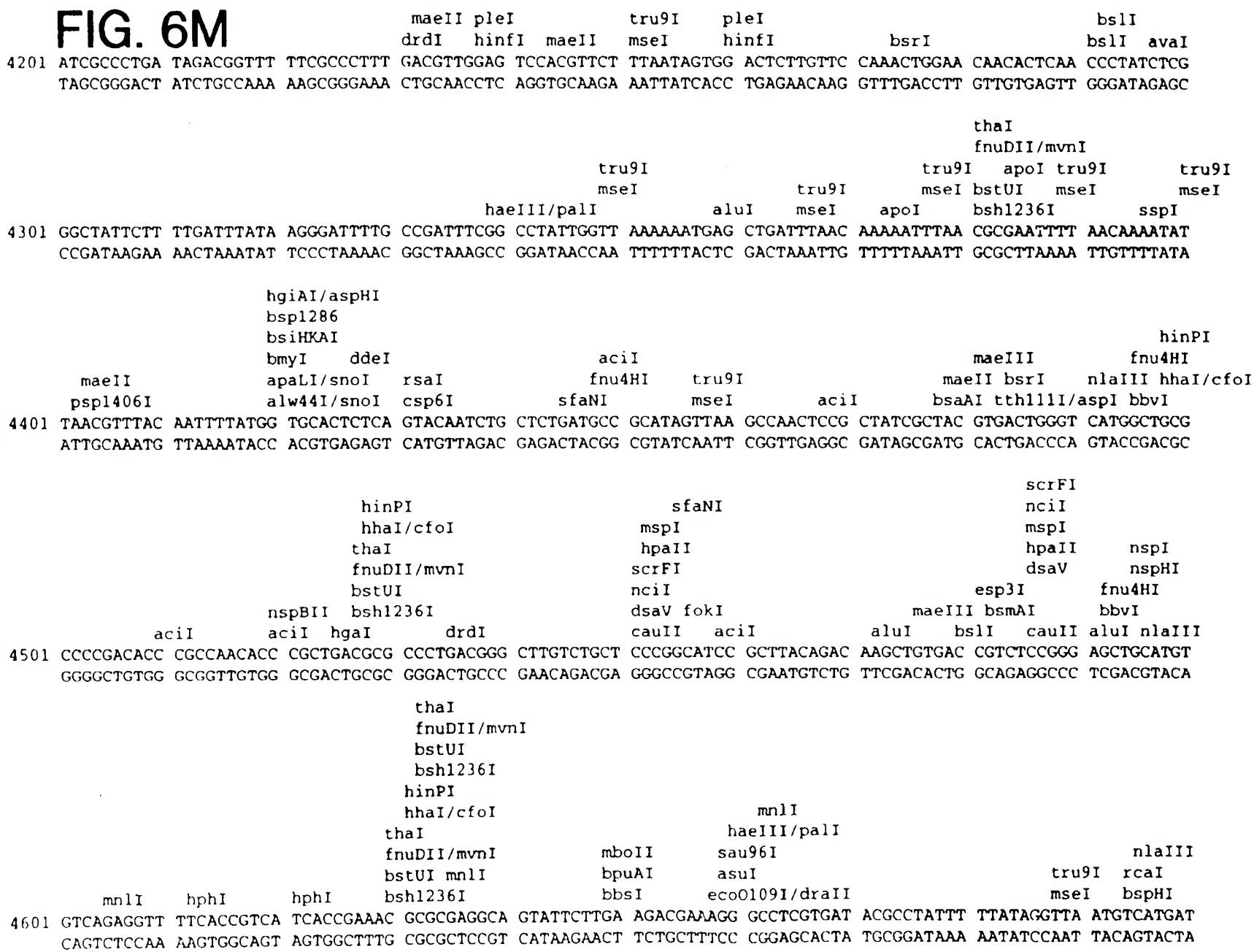

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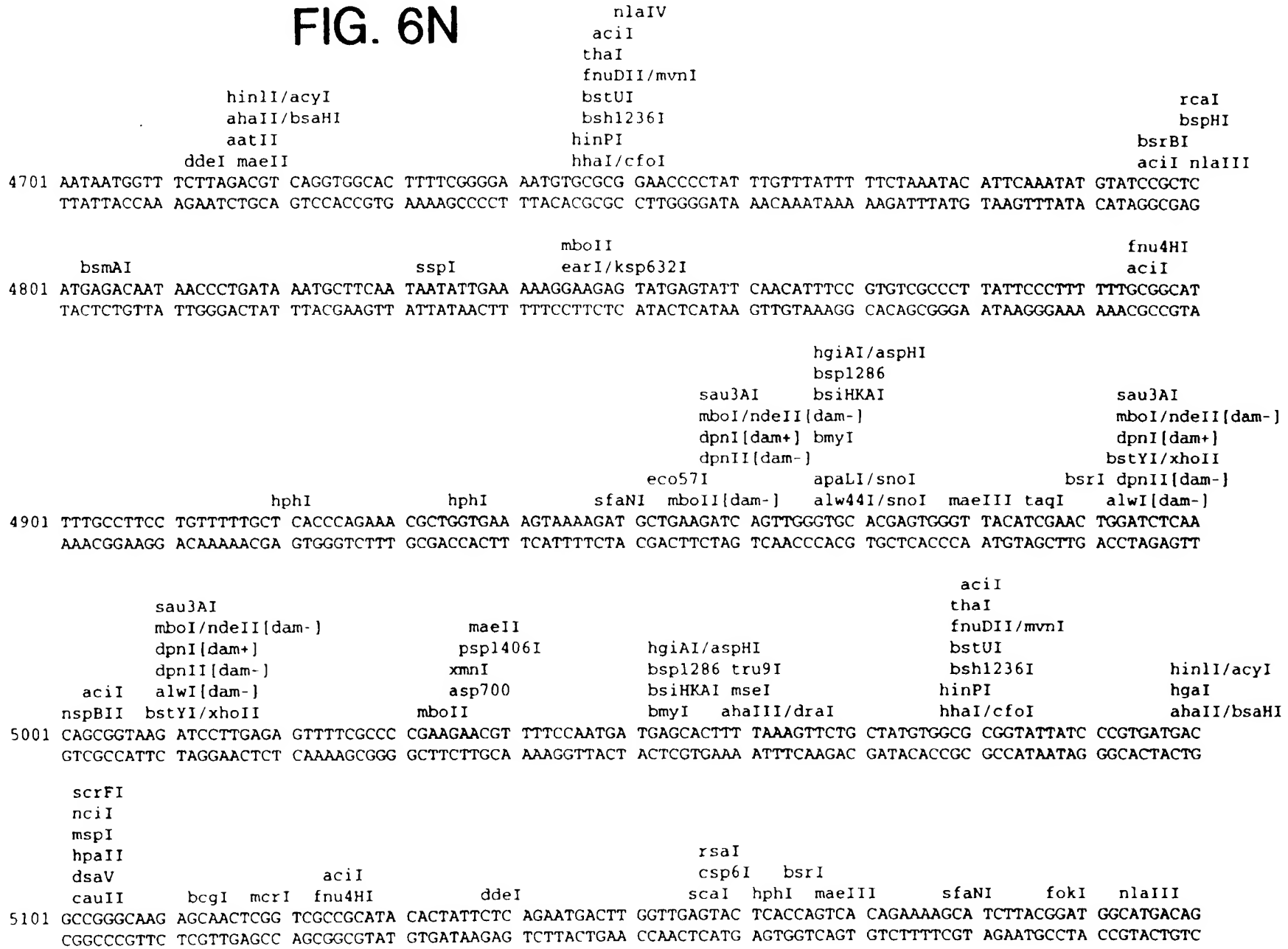
FIG. 6N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aciI
thaI
fnuDII/mvnI
hinlI/acyI
ahaII/bsaHI
aatII
bstUI
bsh1236I
hinPI
rcaI
bspHI
bsrBI
ddeI maeII
hhaI/cfoI
aciI nlaIII
4701 AATAATGGTT TCTTAGACGT CAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC ATTCAAATAT GTATCCGCTC
TTATTACCAA AGAATCTGCA GTCCACCGTG AAAAGCCCCT TTACACGCGC CTTGGGGATA AACAAATAAA AAGATTTATG TAAGTTTATA CATAGGCGAG
mboII
fnu4HI
bsmAI
sspI
earI/ksp632I
aciI
4801 ATGAGACAAT AACCCTGATA AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT
TACTCTGTTA TTGGGACTAT TTACGAAGTT ATTATAACTT TTTCCTTCTC ATACTCATAA GTTGTAAAGG CACAGCGGGA ATAAGGGAAA AAACGCCGTA
hgiAI/aspHI
bsp1286
sau3AI
bsiHKAI
sau3AI
mboI/ndeII[dam-]
mboI/ndeII[dam-]
dpnI[dam+]
bmyI
dpnI[dam+]
dpnII[dam-]
bstYI/xhoII
eco57I
apaLI/snoI
bsrI dpnII[dam-]
hphI
hphI
sfaNI
mboII[dam-]
alw44I/snoI
maeIII taqI
alwI[dam-]
4901 TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA
AAACGGAAGG ACAAAAACGA GTGGGTCTTT GCGACCACTT TCATTTTCTA CGACTTCTAG TCAACCCACG TGCTCACCCA ATGTAGCTTG ACCTAGAGTT
aciI
thaI
sau3AI
fnuDII/mvnI
mboI/ndeII[dam-]
maeII
dpnI[dam+]
psp1406I
hgiAI/aspHI
bstUI
dpnII[dam-]
xmnI
bsp1286 tru9I
bsh1236I
hinlI/acyI
aciI
alwI[dam-]
asp700
bsiHKAI mseI
hinPI
hgaI
nspBII
bstYI/xhoII
mboII
bmyI
ahaIII/draI
hhaI/cfoI
ahaII/bsaHI
5001 CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGATGAC
GTCGCCATTC TAGGAACTCT CAAAAGCGGG GCTTCTTGCA AAAGGTTACT ACTCGTGAAA ATTTCAAGAC GATACACCGC GCCATAATAG GGCACTACTG
scrFI
nciI
mspI
hpaII
rsaI
dsaV
aciI
csp6I
bsrI
cauII
bcgI
mcrI
fnu4HI
ddeI
scaI
hphI
maeIII
sfaNI
fokI
nlaIII
5101 GCCGGGCAAG AGCAACTCGG TCGCCGCATA CACTATTCTC AGAATGACTT GGTTGAGTAC TCACCAGTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG
CGGCCCGTTC TCGTTGAGCC AGCGGCGTAT GTGATAAGAG TCTTACTGAA CCAACTCATG AGTGGTCAGT GTCTTTTCGT AGAATGCCTA CCGTACTGTC

FIG. 6O

```
                                                                                                                    sau96I
                                                                                                                    avaII
                                                                                                          sau3AI    asuI
                                                                                haeIII/palI               mboI/ndeII[dam-]
                                                                               eaeI                       dpnI[dam+]
                                                                               cfrI                       dpnII[dam-]
                                fnu4HI                                        fnu4HI                    pvuI/bspCI
                                bbvI          nlaIII                          aciI                      mcrI    mnlI          aluI    aciI
5201 TAAGAGAATT ATGCAGTGCT GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT CGGAGGACCG AAGGAGCTAA CCGCTTTTTT
     ATTCTCTTAA TACGTCACGA CGGTATTGGT ACTCACTATT GTGACGCCGG TTGAATGAAG ACTGTTGCTA GCCTCCTGGC TTCCTCGATT GGCGAAAAAA

                                maeIII
                              nlaIII
                          sau3AI                                  mspI
                          mboI/ndeII[dam-]      sau3AI  nlaIV
                          dpnI[dam+]            mboI/ndeII[dam-] aluI
                          dpnII[dam-]           dpnI[dam+]   hpaII                                                      fnu4HI
                    nlaIII alwI[dam-]           dpnII[dam-] bsaWI                                  maeIII     sfaNI    bbvI
5301 GCACAACATG GGGGATCATG TAACTCGCCT TGATCGTTGG GAACCGGAGC TGAATGAAGC CATACCAAAC GACGAGCGTG ACACCACGAT GCCAGCAGCA
     CGTGTTGTAC CCCCTAGTAC ATTGAGCGGA ACTAGCAACC CTTGGCCTCG ACTTACTTCG GTATGGTTTG CTGCTCGCAC TGTGGTGCTA CGGTCGTCGT

                                                                        mspI
                          hinPI                                         hpaII
                          mstI                                          scrFI
                          aviII/fspI     bsrI                    aluI  nciI        tru9I      fokI                    sau96I
                      maeII hhaI/cfoI  tru9I                     rmaI  dsaV        mseI    bsrI        aciI            avaII
                     psp1406I          mseI                      maeI  cauII      aseI/asnI/vspI   mnlI               asuI
5401 ATGGCAACAA CGTTGCGCAA ACTATTAACT GGCGAACTAC TTACTCTAGC TTCCCGGCAA CAATTAATAG ACTGGATGGA GGCGGATAAA GTTGCAGGAC
     TACCGTTGTT GCAACGCGTT TGATAATTGA CCGCTTGATG AATGAGATCG AAGGGCCGTT GTTAATTATC TGACCTACCT CCGCCTATTT CAACGTCCTG

                          bglI                                          mspI              thaI
                          sau96I                                        hpaII             fnuDII/mvnI              haeIII/palI
                          haeIII/palI                                   cfr10I            bstUI                   sau96I
                   hinPI  asuI     mspI                              nlaIV hphI        bsmAI aciI        fnu4HI   nlaIV
                   hhaI/cfoI       hpaII                          gsuI/bpmI          bsaI  bsh1236I     bbvI bsrI asuI
5501 CACTTCTGCG CTCGGCCCTT CCGGCTGGCT GGTTTATTGC TGATAAATCT GGAGCCGGTG AGCGTGGGTC TCGCGGTATC ATTGCAGCAC TGGGGCCAGA
     GTGAAGACGC GAGCCGGGAA GGCCGACCGA CCAAATAACG ACTATTTAGA CCTCGGCCAC TCGCACCCAG AGCGCCATAG TAACGTCGTG ACCCCGGTCT

                                                                                    ddeI
                                                                              sau3AI        nlaIV
                                                  pleI                        mboI/ndeII[dam-]  mnlI
                                                  hinfI                       dpnI[dam+]    hgiCI          tru9I
                   mnlI                        eam1105I          fokI         dpnII[dam-]   banI           mseI
5601 TGGTAAGCCC TCCCGTATCG TAGTTATCTA CACGACGGGG AGTCAGGCAA CTATGGATGA ACGAAATAGA CAGATCGCTG AGATAGGTGC CTCACTGATT
     ACCATTCGGG AGGGCATAGC ATCAATAGAT GTGCTGCCCC TCAGTCCGTT GATACCTACT TGCTTTATCT GTCTAGCGAC TCTATCCACG GAGTGACTAA
```


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FIG. 6P

```
                                                                                  rmaI      sau3AI
                                                                           sau3AI hphI  mboI/ndeII[dam-]
                                                                           mboI/ndeII[dam-]
                                                                           dpnI[dam+]   dpnI[dam+]
                                                                           dpnII[dam-]  dpnII[dam-]
                                                   tru9I         ahaIII/draI   maeI     alwI[dam-]
                                                   mseI       tru9I tru9I bstYI/xhoII  bstYI/xhoII
          maeIII                                  ahaIII/draI mseI  mseI  alwI[dam-] mboII[dam-]
5701 AAGCATTGGT AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAAACTT CATTTTTAAT TTAAAAGGAT CTAGGTGAAG ATCCTTTTTG
     TTCGTAACCA TTGACAGTCT GGTTCAAATG AGTATATATG AAATCTAACT AAATTTTGAA GTAAAAATTA AATTTTCCTA GATCCACTTC TAGGAAAAAC

                                                                            sau3AI
                                                                            mboI/ndeII[dam-]
                                                                            dpnI[dam+]  sau3AI
                                                                            dpnII[dam-] mboI/ndeII[dam-]
                                                                           bstYI/xhoII  dpnI[dam+]
                                                                   sau3AI  alwI[dam-]   dpnII[dam-]
         nlaIII           maeII                                    mboI/ndeII[dam-]     alwI[dam-]
        rcaI            tru9I                  hgaI                dpnI[dam+] mboII[dam-]
        bspHI           mseI                ddeI                   dpnII[dam-]        bstYI/xhoII
5801 ATAATCTCAT GACCAAAATC CCTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT
     TATTAGAGTA CTGGTTTTAG GGAATTGCAC TCAAAAGCAA GGTGACTCGC AGTCTGGGGC ATCTTTTCTA GTTTCCTAGA AGAACTCTAG GAAAAAAAGA

                                                              sau3AI
      thaI                                                    mboI/ndeII[dam-]
      fnuDII/mvnI                                             dpnI[dam+]
      bstUI                                                   dpnII[dam-]
      bsh1236I                                               alwI[dam-]
     hinPI    fnu4HI                       aciI          mspI                              bsrI
     hhaI/cfoI bbvI                 aciI  nspBII         hpaII      aluI                maeIII  eco57I
5901 GCGCGTAATC TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTTCCGAA GGTAACTGGC
     CGCGCATTAG ACGACGAACG TTTGTTTTTT TGGTGGCGAT GGTCGCCACC AAACAAACGG CCTAGTTCTC GATGGTTGAG AAAAAGGCTT CCATTGACCG

           hinPI                  rmaI           haeIII/palI
           hhaI/cfoI              maeI     bslI  haeI                scfI   aciI      mnlI
6001 TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC
     AAGTCGTCTC GCGTCTATGG TTTATGACAG GAAGATCACA TCGGCATCAA TCCGGTGGTG AAGTTCTTGA GACATCGTGG CGGATGTATG GAGCGAGACG
```


BNSDOCID <WO __9604391A1_I_>

FIG. 6Q

```
                                                         scrFI                                       aciI
                                                         nciI                                       nspBII
                      fnu4HI                             mspI                        mspI          fnu4HI
             alwNI    bbvI                               hpaII                       hpaII         bbvI
            bsrI  fnu4HI                                 dsaV     pleI              bsaWI        hinPI mcrI
       maeIII     bbvI    bsrI                           cauII    hinfI          maeIII          hhaI/cfoI
6101 TAATCCTGTT ACCAGTGGCT GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT AAGGCGCAGC GGTCGGGCTG
     ATTAGGACAA TGGTCACCGA CGACGGTCAC CGCTATTCAG CACAGAATGG CCCAACCTGA GTTCTGCTAT CAATGGCCTA TTCCGCGTCG CCAGCCCGAC

                  hgiAI/aspHI
                  bsp1286
                  bsiHKAI
                  bmyI                                                                               hinPI
                  apaLI/snoI                                                                         hhaI/cfoI
                  alw44I/snoI   aluI                          ddeI      scfI                        haeII
6201 AACGGGGGGT TCGTGCACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG AACTGAGATA CCTACAGCGT GAGCATTGAG AAAGCGCCAC GCTTCCCGAA
     TTGCCCCCCA AGCACGTGTG TCGGGTCGAA CCTCGCTTGC TGGATGTGGC TTGACTCTAT GGATGTCGCA CTCGTAACTC TTTCGCGGTG CGAAGGGCTT

                                                                               scrFI
                                                                               mvaI          scrFI
                                                                               ecoRII mvaI
                        mspI                                                   dsaV   ecoRII
                        hpaII                                                  bstNI        dsaV
                        bslI  fnu4HI                         hinPI mnlI        bsaJI        bstNI
            aciI       bsaWI  aciI                           hhaI/cfoI   aluI  apyI(dcm+)   apyI(dcm+)
6301 GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCACGAGG GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG
     CCCTCTTTCC GCCTGTCCAT AGGCCATTCG CCGTCCCAGC CTTGTCCTCT CGCGTGCTCC CTCGAAGGTC CCCCTTTGCG GACCATAGAA ATATCAGGAC

                                                                                               haeIII/palI
                                                                                             fnu4HI
                                                                                             aciI
                                                                                            thaI bslI
                                                                                            fnuDII/mvnI
                              taqI                                   nlaIV                  bstUI
             mnlI drdI     hgaI          sfaNI                     aciI                     bsh1236I    nlaIV
6401 TCGGGTTTCG CCACCTCTGA CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC AACGCGGCCT TTTTACGGTT
     AGCCCAAAGC GGTGGAGACT GAACTCGCAG CTAAAAACAC TACGAGCAGT CCCCCCGCCT CGGATACCTT TTTGCGGTCG TTGCGCCGGA AAAATGCCAA
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BNSDOCID <WO__9604391A1_I_>

FIG. 6R

```
          haeIII/palI
         haeI
       scrFI
       mvaI bslI
       ecoRII                  nlaIII
       dsaV                   nspI
       bstNI          haeIII/palI  nspHI                          tfiI                                                    bsrBI
       apyI[dcm+]     haeI         aflIII                         hinfI                    aciI          aluI     aciI
6501   CCTGGCCTTT TGCTGGCCTT TTGCTCACAT GTTCTTTCCT GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT
       GGACCGGAAA ACGACCGGAA AACGAGTGTA CAAGAAAGGA CGCAATAGGG GACTAAGACA CCTATTGGCA TAATGGCGGA AACTCACTCG ACTATGGCGA

                                                                                                thaI
                                                                                                fnuDII/mvnI
                                                                                                bstUI
                                                                                                bsh1236I
                                                                                               hinPI
                                                                                               hhaI/cfoI
                                                                                              thaI
                                                                                              fnuDII/mvnI
          fnu4HI              fnu4HI                         sapI  hinPI                      bstUI   haeIII/palI
          bbvI                bbvI pleI                      mboII hhaI/cfoI                  bsh1236I          tru9I
        aciI                  hinPI  hinfI                   earI/ksp632I                mnlI   bslI     eaeI  tfiI aseI/asnI/vspI
       fnu4HI        mcrI     hhaI/cfoI           mnlI   aciI    haeII            aciI       aciI     cfrI  hinfI mseI
6601   CGCCGCAGCC GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA AGAGCGCCCA ATACGCAAAC CGCCTCTCCC CGCGCGTTGG CCGATTCATT
       GCGGCGTCGG CTTGCTGGCT CGCGTCGCTC AGTCACTCGC TCCTTCGCCT TCTCGCGGGT TATGCGTTTG GCGGAGAGGG GCGCGCAACC GGCTAAGTAA

                                                                                                scrFI
                                                                                                mvaI
                                                                                                ecoRII
                                                                                                dsaV
           aluI                                                    tru9I                        nlaIV bstNI
           pvuII                                          hinPI       mseI      maeIII          hgiCI apyI[dcm+]
           nspBII                        bsrI     aciI    hhaI/cfoI  aseI/asnI/vspI  mnlI       banI bsaJI
6701   AATCCAGCTG GCACGACAGG TTTCCCGACT GGAAAGCGGG CAGTGAGCGC AACGCAATTA ATGTGAGTTA CCTCACTCAT TAGGCACCCC AGGCTTTACA
       TTAGGTCGAC CGTGCTGTCC AAAGGGCTGA CCTTTCGCCC GTCACTCGCG TTGCGTTAAT TACACTCAAT GGAGTGAGTA ATCCGTGGGG TCCGAAATGT

                                                                                                tru9I
                                                                                                mseI
                                                                                               aseI/asnI/vspI
                  mspI                          aciI                                          xmnI
                  hpaII                        bsrBI                              aluI      nlaIII    asp700
6801   CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTAA
       GAAATACGAA GGCCGAGCAT ACAACACACC TTAACACTCG CCTATTGTTA AAGTGTGTCC TTTGTCGATA CTGGTACTAA TGCTTAATT
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>length: 6889

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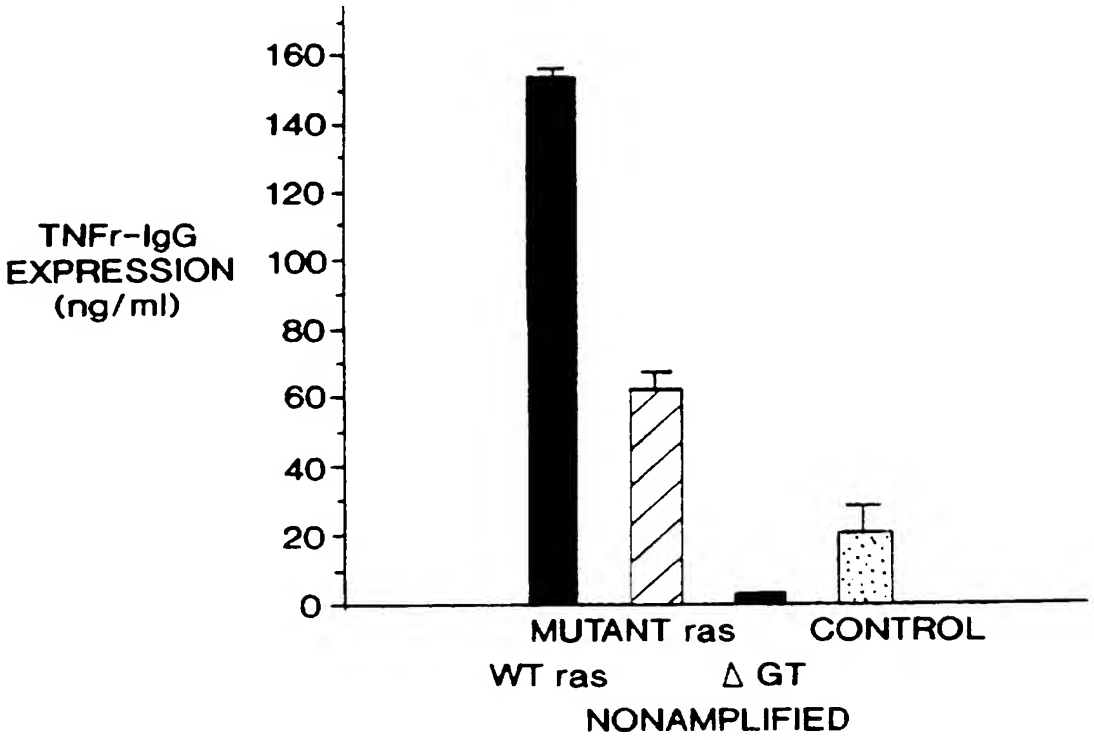

FIG. 7A

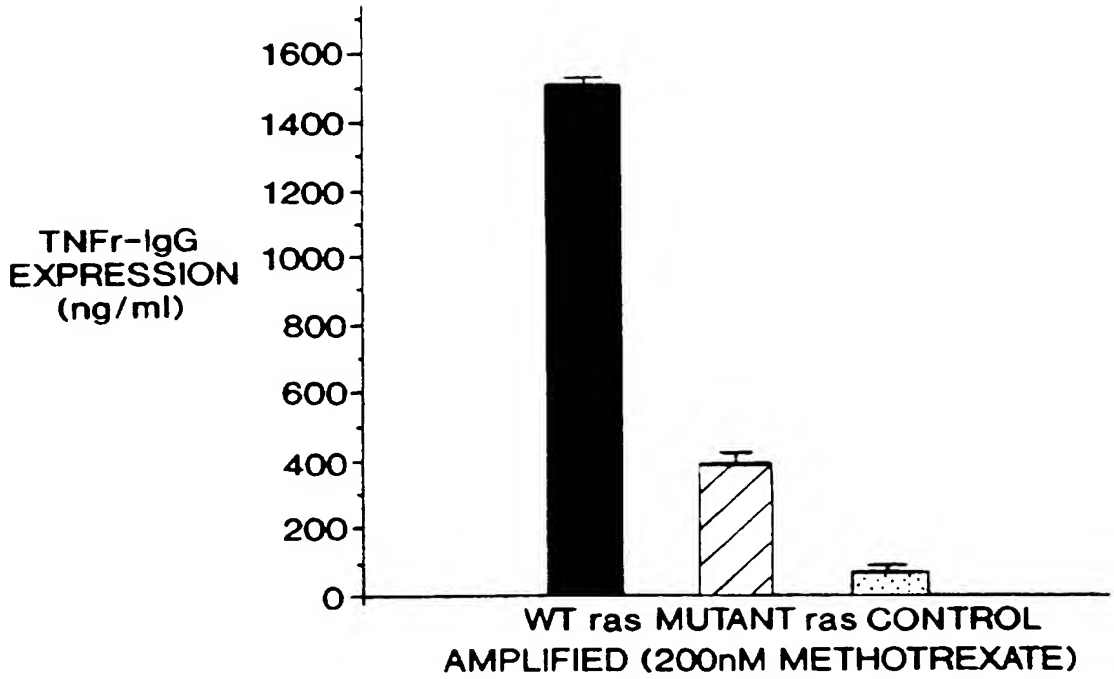

FIG. 7B

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FIG. 8

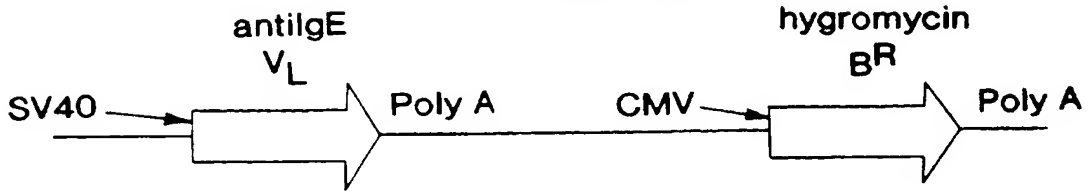

FIG. 11

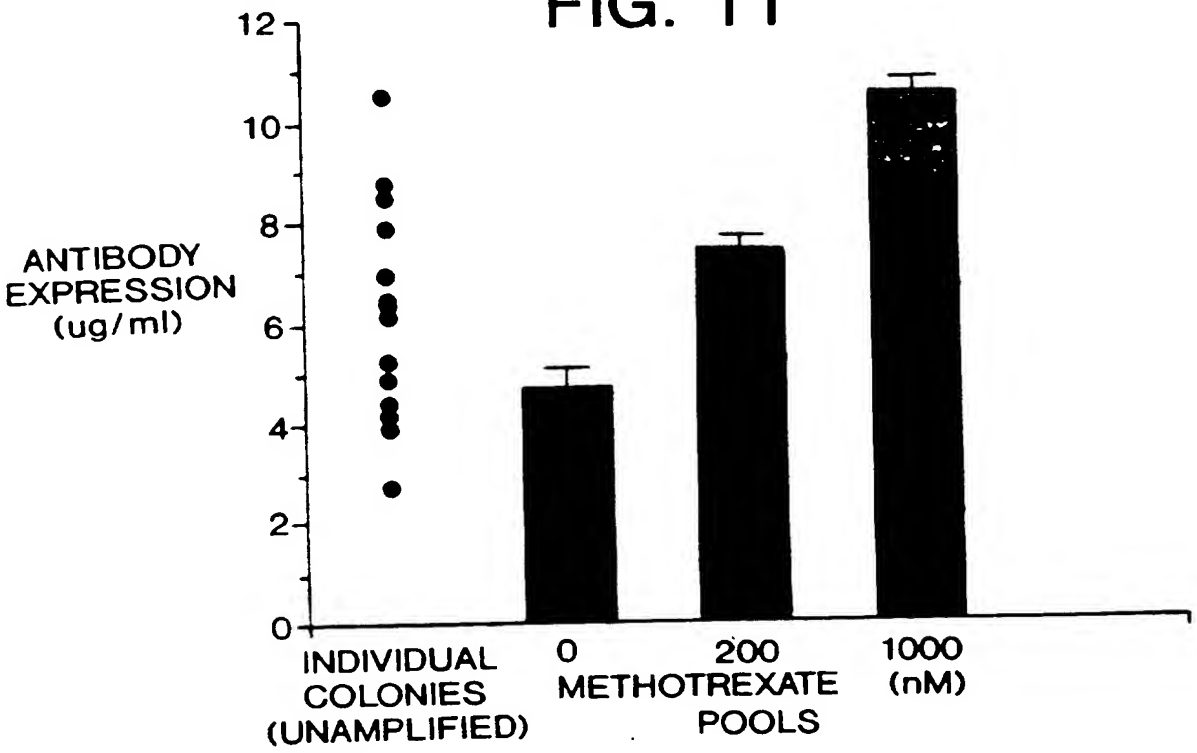

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FIG. 9A

```
                aluI
                sstI
                sacI
                hgiJII
                hgiAI/aspHI
                ecl136II
                bsp1286
                bsiHKAI
                bmyI
                banII
              taqI                                  aluI
                                                    sau3AI pvuII
                                                    mboI/ndeII[dam-]
                                                    dpnI[dam+]
                                                    pvuI/bspCI
                                                  pleI dpnII[dam-]
                                                  hinfI  taqI[dam-]
                                                rmaI    mcrI    nspBII
                                                maeI   taqI[dam-]                                  nlaIV
                                                                                                   scrFI
                                                                                                   mvaI
                                                                                                   ecoRII
                                                                                                   dsaV
                                                                                                   bstNI
                                                                                                   apyI[dcm+]
                                                                                                 bsaJI
1   TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCTCAGCTAG CTGTCGACAC CTTACACACA GTCAATCCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT

                                                                                  nlaIV
                  sfaNI                 scrFI          scrFI                                  sfaNI
                 ppu10I                 mvaI                 mvaI                                 ppu10I
                 nsiI/avaIII            ecoRII         ecoRII                               nsiI/avaIII
                nlaIII                  dsaV           dsaV                                nlaIII
               sphI                     bstNI                bstNI                                sphI
               nspI                     apyI[dcm+]           apyI[dcm+]                           nspI
               nspHI                  sexAI                bsaJI                                  nspHI
101 GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA
    CTTCATACGT TTCGTACGTA GAGTTAATCA GTCGTTGGTC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG TAGAGTTAAT

                                                                                                  nlaIII
                                                                                                  styI
                                                                                                  ncoI
                                                                                              bslI dsaI
                                                  aciI                                        aciI bsaJI
                    aciI          aciI  fokI              aciI bsrI  aciI
201 GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT
    CAGTCGTTGG TATCAGGGCG GGGATTGAGG CGGGTAGGGC GGGGATTGAG GCGGGTCAAG GCGGGTAAGA GGCGGGGTAC CGACTGATTA AAAAAAATAA

                                                                                    rmaI
                                                                                    styI
                                                                                    bsaJI                                  haeIII/palI
                                                                                    blnI                                   mcrI
                                                                                    avrII                        aluI      eagI/xmaIII/eclXI
                                                                                  haeIII/palI                    rmaI      eaeI
                                                                                  stuI                           maeI      cfrI
                                                                                  haeI                           nheI      mspI
                                                                                mnlI maeI                        aluI      hpaII
                  fnu4HI
                  bglI
                 sfiI
                 haeIII/palI
               mnlI   mnlI      ddeI
            haeIII/palI bsaJI  mnlI   aluI                    mnlI
          mnlI bsaJI aciI    haeIII/palI                    mnlI
301 TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG
    ATACGTCTCC GGCTCCGGCG GAGCCGGAGA CTCGATAAGG TCTTCATCAC TCCTCCGAAA AAACCTCCGG ATCCGAAAAC GTTTTTCGAT CGAATAGGCC
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FIG. 9B

```
                                tfiI
    scrFI                       hinfI
    nciI                   aciI
    mspI                   thaI                                                    fnu4HI
    hpaII                  fnuDII/mvnI                  aciI                       bbvI
    dsaV                   bstUI                   maeII  rsaI                    nspBII
    cauII                  bsh1236I               maeIII    csp6I  scfI          mnlI        aciI       nlaIII
401 CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT
    GGCCCTTGCC ACGTAACCTT GCGCCTAAGG GGCACGGTTC TCACTGCATT CATGGCGGAT ATCTCGCTAT TCTCCTAAAA TAGGGGCGAC GGTAGTACCA

                                                                haeIII/palI
                                                               haeI
                                                          scrFI
                                                          mvaI      bsrBI
                                                          ecoRII
                                                          dsaV
                                                          bstNI     aciI                    rsaI
                                      pflMI                       bsmAI     apyI[dcm+]          xmnI          csp6I
    taqI    .       sfaNI             bslI                        bsaI      bsaJI  mnlI  ddeI   asp700        scaI
501 TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA AGAACGGAGA CCTACCCTGG CCTCCGCTCA GGAACGAGTT CAAGTACTTC
    AGCTGGTAAC TTGACGTAGC AGCGGCACAG GGTTTTATAC CCCTAACCGT TCTTGCCTCT GGATGGGACC GGAGGCGAGT CCTTGCTCAA GTTCATGAAG

                                                          scrFI
                                                          mvaI
                                                          ecoRII
                                                          dsaV
                        eco57I                            bstNI                          tfiI       tru9I
                       mboII                tfiI          apyI[dcm+]                      hinfI      mseI
                      earI/ksp632I          hinfI                                  ddeI mboII taqI   ahaIII/draI
                     mnlI                 alwNI    hphI                sexAI
601 CAAAGAATGA CCACAACCTC TTCAGTGGAA GGTAAACAGA ATCTGGTGAT TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA
    GTTTCTTACT GGTGTTGGAG AAGTCACCTT CCATTTGTCT TAGACCACTA ATACCCATCC TTTTGGACCA AGAGGTAAGG ACTCTTCTTA GCTGGAAATT

                                                    sstI
                                                    sacI
                                                    hgiJII
                                                    hgiAI/aspHI
                                                    ecl136II
                                                    bsp1286
                                                    bsiHKAI
          tru9I                                     bmyI                                  tru9I
          mseI          ddeI                        banII                                 aflII/bfrI
         aseI/asnI/vspI                        bslI   mnlI aluI          bstXI      fokI sfaNI mseI
701 AGGACAGAAT TAATATAGTT CTCAGTAGAG AACTCAAAGA ACCACCACGA GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA
    TCCTGTCTTA ATTATATCAA GAGTCATCTC TTGAGTTTCT TGGTGGTGCT CCTCGAGTAA AAGAACGGTT TTCAAACCTA CTACGGAATT CTGAATAACT
```

FIG. 9C

```
                                                                                          haeIII/palI
                                                                                         haeI
                                                             scrFI         scrFI
                                                             mvaI                mvaI
                                                             ecoRII        ecoRII
                                                             dsaV   tfiI   dsaV
       mspI                                                  bstNI   nlaIII      bstNI      ddeI pleI
       hpaII                                                 apyI[dcm+]   hinfI  apyI[dcm+]      hinfI
      bsaWI              accI nlaIII            mnlI
801 ACAACCGGAA TTGGCAAGTA AAGTAGACAT GGTTTGGATA GTCGGAGGCA GTTCTGTTTA CCAGGAAGCC ATGAATCAAC CAGGCCACCT TAGACTCTTT
    TGTTGGCCTT AACCGTTCAT TTCATCTGTA CCAAACCTAT CAGCCTCCGT CAAGACAAAT GGTCCTTCGG TACTTAGTTG GTCCGGTGGA ATCTGAGAAA

                                                                                               hgaI
                                                                                              hinlI/acyI
                                                                                              ahaII/bsaHI
                                                                                            scrFI
           nlaIII                                                                           mvaI     mnlI
         sau3AI                                                                             ecoRII
         mboI/ndeII[dam-]                                                                   dsaV
         dpnI[dam+]                  maeII                                                  bstNI    ecoNI
         dpnII[dam-]                aflIII                                                  apyI[dcm+]    mnlI
maeIII alwI[dam-]  apoI          maeIII                                       mnlI         bsaJI     bslI ddeI
901 GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCCAGAAAT TGATTTGGGG AAATATAAAC CTCTCCCAGA ATACCCAGGC GTCCTCTCTG
    CACTGTTCCT AGTACGTCCT TAAACTTTCA CTGTGCAAAA AGGGTCTTTA ACTAAACCCC TTTATATTTG GAGAGGGTCT TATGGGTCCG CAGGAGAGAC

       scrFI
       mvaI
       ecoRII
       dsaV
       bstNI
       apyI[dcm+]
     sau96I
     avaII                                                         sfaNI
     asuI   mnlI      sfaNI             accI      mboII              mboII                     mnlI      aluI
1001 AGGTCCAGGA GGAAAAAGGC ATCAAGTATA AGTTTGAAGT CTACGAGAAG AAAGACTAAC AGGAAGATGC TTTCAAGTTC TCTGCTCCCC TCCTAAAGCT
     TCCAGGTCCT CCTTTTTCCG TAGTTCATAT TCAAACTTCA GATGCTCTTC TTTCTGATTG TCCTTCTACG AAAGTTCAAG AGACGAGGGG AGGATTTCGA
                                                  styI
                                                  bsaJI
                                             sau3AI
                         nlaIII              mboI/ndeII[dam-]
                        styI                 dpnI[dam+]
                        ncoI                 dpnII[dam-]              aluI     tru9I
     ppu10I             dsaI                 alwI[dam-]              fnu4HI    mseI
     nsiI/avaIII        bsaJI               bstYI/xhoII              bbvI     aseI/asnI/vspI
1101 ATGCATTTTT ATAAGACCAT GGGACTTTTG CTGGCTTTAG ATCCCCTTGG CTTCGTTAGA ACGCAGCTAC AATTAATACA TAACCTTATG TATCATACAC
     TACGTAAAAA TATTCTGGTA CCCTGAAAAC GACCGAAATC TAGGGGAACC GAAGCAATCT TGCGTCGATG TTAATTATGT ATTGGAATAC ATAGTATGTG
```


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FIG. 9D

```
                                                                                                  sau96I
                                                                                                  avaII
                                                                                                  asuI
                                                                                              scrFI
                                                                                              mvaI
                                                                                              ecoRII
                                                                                              dsaV
                                                                                              bstNI                                    ecoRI
                    maeIII                                                                    apyI[dcm+]      mnlI        taqI   apoI
                   hphI   scfI       fokI                                          bslI  bsaJI            bsaJI      claI/bsp106
1201 ATACGATTTA GGTGACACTA TAGATAACAT CCACTTTGCC TTTCTCTCCA CAGGTGTCCA CTCCCAGGTC CAACTGCACC TCGGTTCTAT CGATTGAATT
     TATGCTAAAT CCACTGTGAT ATCTATTGTA GGTGAAACGG AAAGAGAGGT GTCCACAGGT GAGGGTCCAG GTTGACGTGG AGCCAAGATA GCTAACTTAA

                                                                                                                  scrFI
           nlaIII                                                                                                 mvaI
          styI                                                                                                    ecoRII
          pflMI                                                                                                   dsaV
          ncoI                                                                                                    bstNI fnu4HI
          dsaI                                                          rsaI            aluI                      apyI[dcm+]
          bslI fokI                          rmaI             gsuI/bpmI             pvuII     pleI        haeI      bbvI
          bsaJI        nlaIII  fokI          maeI            bsrI   csp6I           nspBII    hinfI   aciI haeIII/palI
1301 CCACCATGGG ATGGTCATGT ATCATCCTTT TTCTAGTAGC AACTGCAACT GGAGTACATT CAGAAGTTCA GCTGGTGGAG TCTGGCGGTG GCCTGGTGCA
     GGTGGTACCC TACCAGTACA TAGTAGGAAA AAGATCATCG TTGACGTTGA CCTCATGTAA GTCTTCAAGT CGACCACCTC AGACCGCCAC CGGACCACGT
```

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FIG. 9E

```
                                                                                                                       scrFI
                                                                                                                       nciI
                                                                                                                       mspI
                                                                                                                       hpaII
                                                                                                                       dsaV
                                                                                                                       cauII
                                                                                                                       bslI
                                                                                                                      xmaI/pspAI
                                                                                                                      smaI
                                                                                                                      scrFI
                                                                                                                      nciI
                                                                                                                      dsaV
                                                                                                                      cauII          scrFI
           hgiJII                                                                                                 sau96I             mvaI
           bsp1286                                                                                      sau3AI    nlaIV              ecoRII
           bmyI                                                                   mspI                  mboI/ndeII[dam-]             dsaV
      scrFI                                                                 hpaII                  dpnI[dam+]  bslI           bstNI
      mvaI banII                                                                 mroI                   dpnII[dam-]  bsaJI           bslI
      ecoRII                                                               bspMII                nlaIV     haeIII/palI        apyI[dcm+]
      dsaV                                                                 bspEI                 bstYI/xhoII   avaI        sau96I
      bstNI                                                                      bsaWI                  bamHI      eco0109I/draII haeIII/palI
      bsaJI                                                                    mnlI                     alwI[dam-] asuI           asuI
      apyI[dcm+]                                   alwNI                   hphI  accIII      aluI   bsrI alwI[dam-]  bsaJI        eco0109I/draII
1401 GCCAGGGGGC TCACTCCGTT TGTCCTGTGC AGTTTCTGGC TACTCCATCA CCTCCGGATA TAGCTGGAAC TGGATCCGTC AGGCCCCGGG TAAGGGCCTG
     CGGTCCCCCG AGTGAGGCAA ACAGGACACG TCAAAGACCG ATGAGGTAGT GGAGGCCTAT ATCGACCTTG ACCTAGGCAG TCCGGGGCCC ATTCCCGGAC

                                           sau3AI
                                           mboI/ndeII[dam-]
                                           dpnI[dam+]                                                      thaI
                                   maeII   dpnII[dam-]                                                     fnuDII/mvnI
                             taqI snaBI   alwI[dam-]                hgaI       haeIII/palI                 bstUI  tfiI
                            claI/bsp106  mspI                    rmaI        sau96I                        bsh1236I
                        sfaNI     bsaAI  hpaII taqI[dam-]         maeI        asuI                        nruI    hinfI
1501 GAATGGGTTG CATCGATTAC GTATGCCGGA TCGACTAACT ATAACCCTAG CGTCAAGGGC CGTATCACTA TAAGTCGCGA CGATTCCAAA AACACATTCT
     CTTACCCAAC GTAGCTAATG CATACGGCCT AGCTGATTGA TATTGGGATC GCAGTTCCCG GCATAGTGAT ATTCAGCGCT GCTAAGGTTT TTGTGTAAGA
```


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FIG. 9F

```
                                                                                       scrFI
                                                                                       mvaI
                                                                                       ecoRII
                                                                                       dsaV
                                                                                       bstNI
                                                                                       apyI[dcm+]
                                                                                    hinPI
                                                                                    hhaI/cfoI
                                                                mnlI               nlaIV
                                                             xhoI                  narI
                                                             paeR7I                kasI
                                                             avaI                  hinlI/acyI
       scfI                                              hgiAI/aspHI               hgiCI
       pstI                                              bsp1286                   haeII
       bsgI                      mnlI                    bsiHKAI   fnu4HI          banI
     bspMI                     ddeI drdI                 bmyI taqI bbvI            ahaII/bsaHI
1601 ACCTGCAGAT GAACAGCCTG CGTGCTGAGG ACACTGCCGT CTATTATTGT GCTCGAGGCA GCCACTATTT CGGCGCCTGG CACTTCGCCG TGTGGGGTCA
     TGGACGTCTA CTTGTCGGAC GCACGACTCC TGTGACGGCA GATAATAACA CGAGCTCCGT CGGTGATAAA GCCGCGGACC GTGAAGCGGC ACACCCCAGT

                                             sau96I
                                             haeIII/palI
                                            sau96I                                                           scrFI
                                            nlaIV                                                            mvaI
                                            hgiJII                nlaIV                                      ecoRII
                                            bsp1286               hgiCI                                      dsaV
           scrFI                      bsp120I                banI                                     bstNI
           mvaI                             bmyI                scrFI                                 bsaJI
           ecoRII                     banII               mvaI                                     sau96I
           dsaV                       asuI                ecoRII                                haeIII/palI
           bstNI hphI                       apaI        mboII   dsaV          hgiAI/aspHI           asuI
           apyI[dcm+] bsmAI    haeIII/palI eco0109I/draII   bstNI               bsp1286              fnu4HI
          bsaJI maeIII     mnlI         styI asuI      bpuAI    apyI[dcm+]  mnlI    bsiHKAI      bsp1286 aciI apyI[dcm+]
      nlaIV   bstEII esp3I bsaJI mnlI   bsaJI          bbsI    bsaJI     mnlI       bmyI mnlI    bmyI nspBII bsaJI
1701 AGGAACCCTG GTCACCGTCT CCTCGGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG
     TCCTTGGGAC CAGTGGCAGA GGAGCCGGAG GTGGTTCCCG GGTAGCCAGA AGGGGGACCG TGGGAGGAGG TTCTCGTGGA GACCCCCGTG TCGCCGGGAC
```


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FIG. 9G

```
          scrFI                                          hinPI
          mvaI                                                nlaIV
          ecoRII                                         narI
          ecoNI                                               kasI                    hgiAI/aspHI
          dsaV                        hphI                hinlI/acyI          bsp1286      mspI
          bstNI                           mspI                  hgiCI                 bsiHKAI      hpaII
          bslI                            hpaII                 haeII                 bmyI         scrFI
          apyI[dcm+]                    cfr10I                  banI                fnu4HI             nciI
        fnu4HI                          bsaWI  tth111I/aspI     ahaII/bsaHI   aciI  apaLI/snoI   dsaV
        bbvI                      bslI  ageI maeIII           ddeI hhaI/cfoI  nspBII  alw44I/snoI cauII      scfI
1801 GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC CTGACCAGCG GCGTGCACAC CTTCCCGGCT GTCCTACAGT
     CCGACGGACC AGTTCCTGAT GAAGGGGCTT GGCCACTGCC ACAGCACCTT GAGTCCGCGG GACTGGTCGC CGCACGTGTG GAAGGGCCGA CAGGATGTCA

                                                        nlaIV
        ddeI pleI                                  fnu4HI   hgiCI
       mnlI  hinfI                            mnlI   bbvI     banI                          tfiI
       eco81I          mnlI  fnu4HI  maeIII bsp1286 rmaI       bsp1286                     hinfI                styI
       bsu36I/mstII/sauI ddeI bbvI   hphI    bmyI    maeI aluI  bmyI                  maeII                      bsaJI
1901 CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACTGTGCC CTCTAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC
     GGAGTCCTGA GATGAGGGAG TCGTCGCACC ACTGACACGG GAGATCGTCG AACCCGTGGG TCTGGATGTA GACGTTGCAC TTAGTGTTCG GGTCGTTGTG

                                                                                    eaml105I
                                                                                   sau96I
                                                                          scrFI
                                                                          mvaI     avaII
                                                                          ecoRII
                          hgiJII                                          dsaV
                          bsp1286                      nlaIII             bstNI    asuI       mboII mboII
                          bmyI                        nspI       bsp1286  bsaJI  nlaIV      bpuAI earI/ksp632I
                          banII          maeIII       nspHI      bmyI  alwNI  apyI[dcm+]       bbsI mnlI
2001 CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT GCCCACCGTG CCCAGCACCT GAACTCCTGG GGGGACCGTC AGTCTTCCTC
     GTTCCACCTG TTCTTTCAAC TCGGGTTTAG AACACTGTTT TGAGTGTGTA CGGGTGGCAC GGGTCGTGGA CTTGAGGACC CCCCTGGCAG TCAGAAGGAG
```

FIG. 9H

```
                                             sau96I
                                             nlaIV
                                             avaII
                                           mspI
                                          scrFI
                                          nciI
                                    sau3AI hpaII
                                    mboI/ndeII[dam-]
                                    dpnI[dam+]                 nlaIII
                                 nlaIII   dsaV         mnlI   nspI                             drdI  mnlI
                                rcaI      cauII      ddeI maeIII                           mboII   ddeI
                   styI         bspHI[dam-]  asuI   eco81I    nspHI                        bpuAI  eco81I
                   bsaJI      mnlI  dpnII[dam-]     bsu36I/mstII/sauI          maeII       bbsI   bsu36I/mstII/sauI
2101 TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA
     AAGGGGGGTT TTGGGTTCCT GTGGGAGTAC TAGAGGGCCT GGGGACTCCA GTGTACGCAC CACCACCTGC ACTCGGTGCT TCTGGGACTC CAGTTCAAGT

                                                        aciI
                                                       thaI
                                                       fnuDII/mvnI
                                                       bstUI
                                                       bsh1236I
                                                      sacII/sstII
                                                      nspBII
                                                      kspI
           maeII                                      dsaI                         rsaI
         rsaI                                         bsaJI                        csp6I
         csp6I                                        aciI           rsaI       maeII                 mnlI      ecoNI
     bsrI bsaAI            mnlI                      fnu4HI  mnlI    csp6I     bsaAI               hgaI   hphI  bslI
2201 ACTGGTACGT GGACGGCGTG GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT ACAACAGCAC GTACCGTGTG GTCAGCGTCC TCACCGTCCT
     TGACCATGCA CCTGCCGCAC CTCCACGTAT TACGGTTCTG TTTCGGCGCC CTCCTCGTCA TGTTGTCGTG CATGGCACAC CAGTCGCAGG AGTGGCAGGA

        scrFI
        mvaI bsrI
        ecoRII
        dsaV
        bstNI                     rsaI            bsmAI                                                     fnu4HI
        apyI[dcm+]                csp6I         bsaI             mnlI           taqI                        bbvI
2301 GCACCAGGAC TGGCTGAATG GCAAGGAGTA CAAGTGCAAG GTCTCCAACA AAGCCCTCCC AGCCCCCATC GAGAAAACCA TCTCCAAAGC CAAAGGGCAG
     CGTGGTCCTG ACCGACTTAC CGTTCCTCAT GTTCACGTTC CAGAGGTTGT TTCGGGAGGG TCGGGGGTAG CTCTTTTGGT AGAGGTTTCG GTTTCCCGTC
```

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BNSDOCID <WO___9604391A1_1_>

FIG. 9I

```
                                                scrFI
                                                nciI
                                                mspI
                                                hpaII
                                                dsaV
                                                cauII
                                               xmaI/pspAI
                                                smaI
                                                scrFI                    scrFI
                                                nciI                     mvaI                scrFI
                                                dsaV                     ecoRII       mvaI
                                                cauII                    dsaV         ecoRII
                                  rsaI          fokI    mboII            bstNI               dsaV
                                  csp6I       bslI  bsaJI                apyI[dcm+]          bstNI
      avaI                      bsp1407I      bslI   avaI earI/ksp632I    sexAI          bspMI apyI[dcm+]
2401  CCCCGAGAAC CACAGGTGTA CACCCTGCCC CCATCCCGGG AAGAGATGAC CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG
      GGGGCTCTTG GTGTCCACAT GTGGGACGGG GGTAGGGCCC TTCTCTACTG GTTCTTGGTC CAGTCGGACT GGACGGACCA GTTTCCGAAG ATAGGGTCGC

            dsaI                        mspI
            bslI                      fnu4HI                                        pleI                 mnlI
            bsaJI                     bbvI hpaII                    mnlI          hinfI     nlaIV   mboII   scfI    aluI
2501  ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA
      TGTAGCGGCA CCTCACCCTC TCGTTACCCG TCGGCCTCTT GTTGATGTTC TGGTGCGGAG GGCACGACCT GAGGCTGCCG AGGAAGAAGG AGATGTCGTT

                                        bpuAI
                                        bbsI                    nlaIII
            dsaI                        maeII                 ppu10I                                sapI
            bsaJI                 fnu4HI    xmnI mboII          nsiI/avaIII                           mboII  mnlI
        hphI              bspMI   bbvI      asp700      nlaIII    sfaNI   mnlI                        earI/ksp632I bslI
2601  GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC
      CGAGTGGCAC CTGTTCTCGT CCACCGTCGT CCCCTTGCAG AAGAGTACGA GGCACTACGT ACTCCGAGAC GTGTTGGTGA TGTGCGTCTT CTCGGAGAGG

                                                              styI
            scrFI                       taqI                aciI
            nciI                        salI               fnu4HI   sau96I
            mspI                    rmaI  hincII/hindII      sfiI ncoI haeIII/palI
            hpaII                   maeI  accI   scfI        haeIII/palI
            dsaV                 sau96I  pleI    pstI       eaeI  dsaI asuI              aluI
            cauII                haeIII/palI     bsgI   aluI cfrI  bsaJI               fnu4HI
        bsmAI                    asuI    hinfI bspMI    hindIII bglI nlaIII             bbvI          maeIII
2701  CTGTCTCCGG GTAAATGAGT GCGACGGCCC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT
      GACAGAGGCC CATTTACTCA CGCTGCCGGG ATCTCAGCTG GACGTCTTCG AACCGGCGGT ACCGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA
```

BNSDOCID: <WO__9604391A1_I_>

FIG. 9J

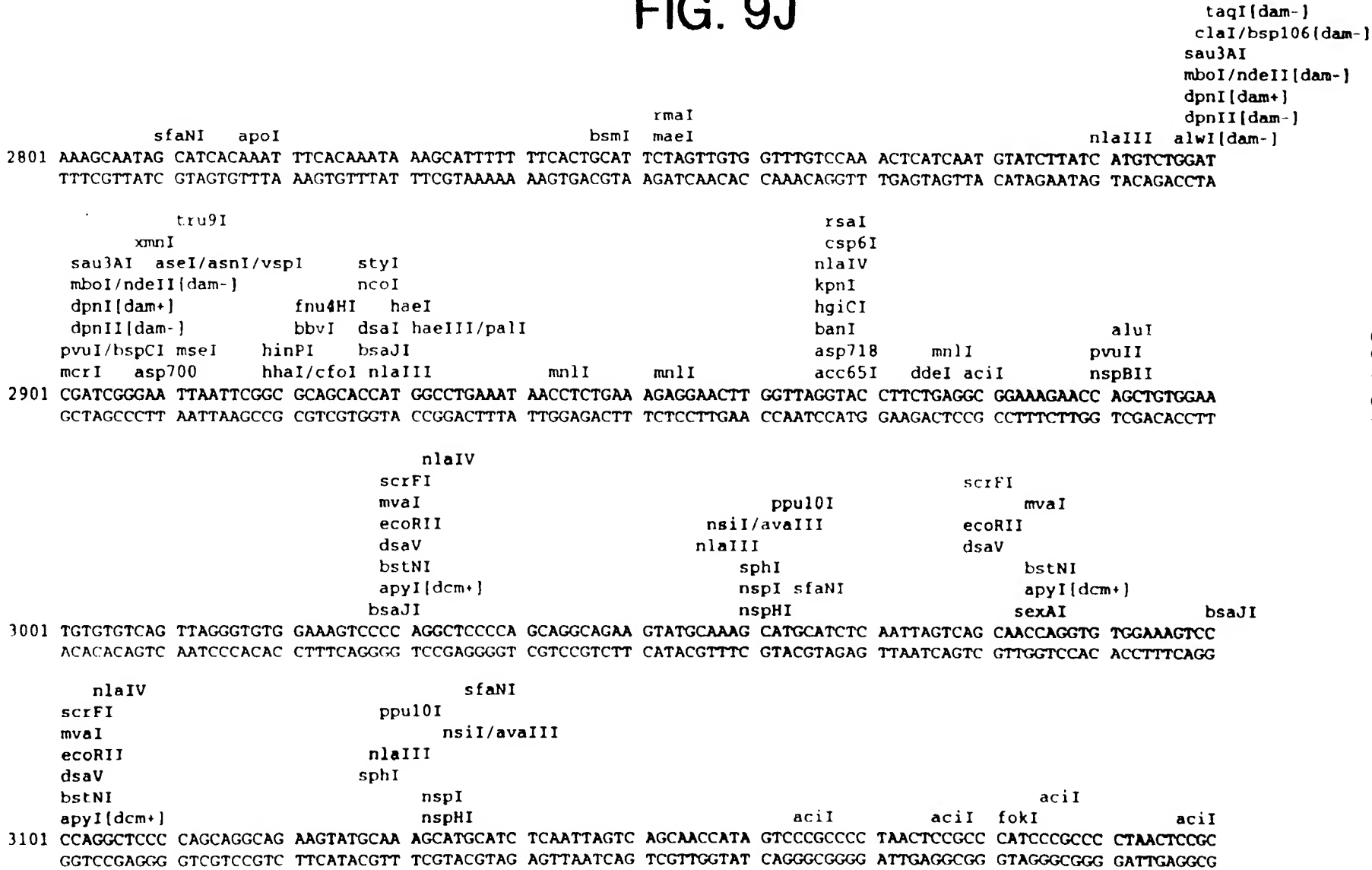

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FIG. 9K

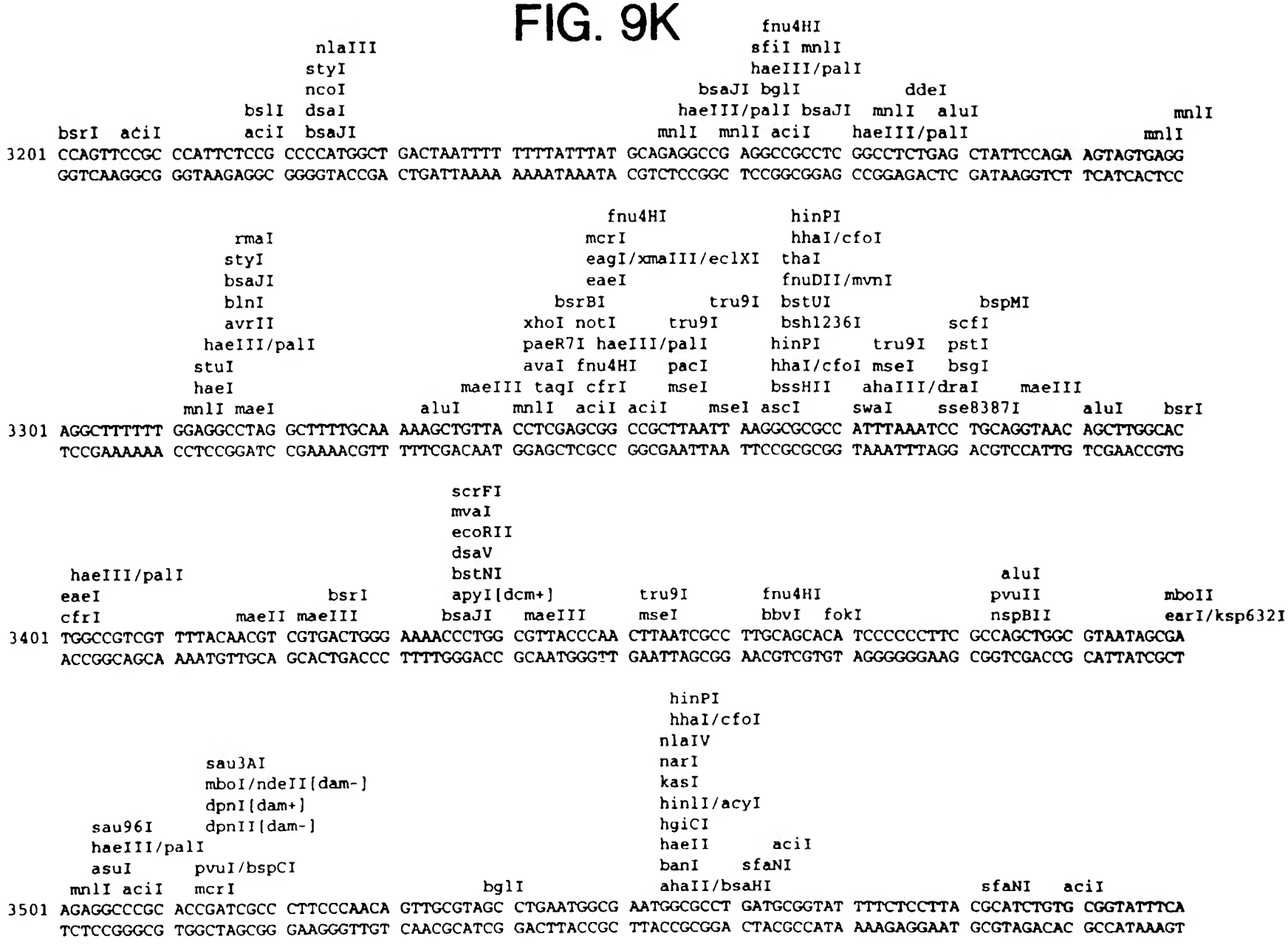

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FIG. 9L

```
                                              aciI
                 hinPI                      fnu4HI                fnu4HI
                 hhaI/cfoI                  aciI                hinPI
                thaI                       thaI                 hhaI/cfoI
                fnuDII/mvnI                fnuDII/mvnI         thaI
                bstUI              hinPI   bstUI               fnuDII/mvnI
                bsh1236I           hhaI/cfoI hinPI             bstUI                            hinPI
              rsaI       scfI fnu4HI  tru9I bsh1236I           bsh1236I      aciI               hhaI/cfoI
 aciI  maeII  csp6I      bslI aciI    mseI hhaI/cfoI       maeIII  bbvI   maeIII              haeII
3601 CACCGCATAC GTCAAAGCAA CCATAGTACG CGCCCTGTAG CGGCGCATTA AGCGCGGCGG GTGTGGTGGT TACGCGCAGC GTGACCGCTA CACTTGCCAG
     GTGGCGTATG CAGTTTCGTT GGTATCATGC GCGGGACATC GCCGCGTAAT TCGCGCCGCC CACACCACCA ATGCGCGTCG CACTGGCGAT GTGAACGGTC

        hinPI                                                                      nlaIV
        hhaI/cfoI                                  mspI                            hgiJII
       haeII                                       hpaII                           bsp1286
      rmaI    bsrBI                               naeI                             bmyI
      maeI    aciI          mboII          maeII  cfr10I           aluI            banII         nlaIV
3701 CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT CCCTTCCTTT CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC
     GCGGGATCGC GGGCGAGGAA AGCGAAAGAA GGGAAGGAAA GAGCGGTGCA AGCGGCCGAA AGGGGCAGTT CGAGATTTAG CCCCCGAGGG AAATCCCAAG

              nlaIV                                 maeII    haeIII/palI
              hgiCI  taqI                          draIII   sau96I                                   maeII
              banI mnlI                    hphI    bsaAI    asuI                                     drdI
3801 CGATTTAGTG CTTTACGGCA CCTCGACCCC AAAAAACTTG ATTTGGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA GACGGTTTTT CGCCCTTTGA
     GCTAAATCAC GAAATGCCGT GGAGCTGGGG TTTTTTGAAC TAAACCCACT ACCAAGTGCA TCACCCGGTA GCGGGACTAT CTGCCAAAAA GCGGGAAACT

       pleI        tru9I     pleI                            bslI
       hinfI  maeII mseI     hinfI         bsrI             bslI   avaI
3901 CGTTGGAGTC CACGTTCTTT AATAGTGGAC TCTTGTTCCA AACTGGAACA ACACTCAACC CTATCTCGGG CTATTCTTTT GATTTATAAG GGATTTTGCC
     GCAACCTCAG GTGCAAGAAA TTATCACCTG AGAACAAGGT TTGACCTTGT TGTGAGTTGG GATAGAGCCC GATAAGAAAA CTAAATATTC CCTAAAACGG

                                                 thaI                                   hgiAI/aspHI
                                                 fnuDII/mvnI                            bsp1286
                                                 bstUI                  maeII           bsiHKAI
              tru9I                            tru9I                  tru9I             bmyI     ddeI
              mseI                tru9I        mseI   apoI tru9I      mseI              apaLI/snoI  rsaI
        haeIII/palI      aluI     mseI   apoI  bsh1236I mseI     sspI psp1406I          alw44I/snoI csp6I
4001 GATTTCGGCC TATTGGTTAA AAAATGAGCT GATTTAACAA AAATTTAACG CGAATTTTAA CAAAATATTA ACGTTTACAA TTTTATGGTG CACTCTCAGT
     CTAAAGCCGG ATAACCAATT TTTTACTCGA CTAAATTGTT TTTAAATTGC GCTTAAAATT GTTTTATAAT TGCAAATGTT AAAATACCAC GTGAGAGTCA
```


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FIG. 9M

```
                                                                                                       hinPI
                                                                                                        hhaI/cfoI
                                                                                                       thaI
                                                                                                       fnuDII/mvnI
                                                                     hinPI                             bstUI
               aciI                               maeIII           fnu4HI                              bsh1236I
              fnu4HI    tru9I                    maeII bsrI   nlaIII  hhaI/cfoI             nspBII     bsh1236I
           sfaNI       mseI          aciI       bsaAI tth111I/aspI bbvI          aciI       aciI  hgaI
4101 ACAATCTGCT CTGATGCCGC ATAGTTAAGC CAACTCCGCT ATCGCTACGT GACTGGGTCA TGGCTGCGCC CCGACACCCG CCAACACCCG CTGACGCGCC
     TGTTAGACGA GACTACGGCG TATCAATTCG GTTGAGGCGA TAGCGATGCA CTGACCCAGT ACCGACGCGG GGCTGTGGGC GGTTGTGGGC GACTGCGCGG

                                                                                                           thaI
                                                                                                           fnuDII/mvnI
                                                                                                           bstUI
                                                          scrFI                                            bsh1236I
                mspI                                      nciI                                            hinPI
                hpaII                                     mspI                                            hhaI/cfoI
               scrFI                                bsmAI          nspI                                  thaI
               nciI    aciI                        esp3I hpaII fnu4HI                                    fnuDII/mvnI
               dsaV sfaNI                         bslI    dsaV aluI nspHI                                bstUI
    drdI       cauII fokI               aluI maeIII       cauII bbvI nlaIII   mnlI   hphI      hphI      bsh1236I
4201 CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC
     GACTGCCCGA ACAGACGAGG GCCGTAGGCG AATGTCTGTT CGACACTGGC AGAGGCCCTC GACGTACACA GTCTCCAAAA GTGGCAGTAG TGGCTTTGCG

                          mnlI
                        haeIII/palI                                                         maeII
               mboII   sau96I                                 nlaIII                       hinlI/acyI
               bpuAI   asuI                           tru9I  rcaI                          ahaII/bsaHI
    mnlI       bbsI   eco0109I/draII                  mseI   bspHI                   ddeI aatII
4301 GCGAGGCAGT ATTCTTGAAG ACGAAAGGGC CTCGTGATAC GCCTATTTTT ATAGGTTAAT GTCATGATAA TAATGGTTTC TTAGACGTCA GGTGGCACTT
     CGCTCCGTCA TAAGAACTTC TGCTTTCCCG GAGCACTATG CGGATAAAAA TATCCAATTA CAGTACTATT ATTACCAAAG AATCTGCAGT CCACCGTGAA

                 nlaIV
               aciI
              thaI
              fnuDII/mvnI
              bstUI                                                    rcaI
              bsh1236I                                                 bspHI
             hinPI                                                  bsrBI    bsmAI
             hhaI/cfoI                                              aciI nlaIII                                sspI
4401 TTCGGGGAAA TGTGCGCGGA ACCCCTATTT GTTTATTTTT CTAAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA CCCTGATAAA TGCTTCAATA
     AAGCCCCTTT ACACGCGCCT TGGGGATAAA CAAATAAAAA GATTTATGTA AGTTTATACA TAGGCGAGTA CTCTGTTATT GGGACTATTT ACGAAGTTAT

            mboII                                                 fnu4HI
            earI/ksp632I                                          aciI                     hphI
4501 ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT GTCGCCCTTA TTCCCTTTTT TGCGGCATTT TGCCTTCCTG TTTTTGCTCA CCCAGAAACG
     TATAACTTTT TCCTTCTCAT ACTCATAAGT TGTAAAGGCA CAGCGGGAAT AAGGGAAAAA ACGCCGTAAA ACGGAAGGAC AAAAACGAGT GGGTCTTTGC
```


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FIG. 9N

```
                                                  hgiAI/aspHI
                                                  bsp1286
                                  sau3AI          bsiHKAI                                 sau3AI  nspBII     sau3AI
                                  mboI/ndeII[dam-]                                        mboI/ndeII[dam-]   mboI/ndeII[dam-]
                                  dpnI[dam+]      bmyI                                    dpnI[dam+]         dpnI[dam+]
                                  dpnII[dam-]                                          bstYI/xhoII           dpnII[dam-]
                          eco57I                  apaLI/snoI                    bsrI dpnII[dam-]           alwI[dam-]
      hphI          sfaNI  mboII[dam-]   alw44I/snoI  maeIII taqI   alwI[dam-]   aciI  bstYI/xhoII                    mboII
4601  CTGGTGAAAG TAAAAGATGC TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG GATCTCAACA GCGGTAAGAT CCTTGAGAGT TTTCGCCCCG
      GACCACTTTC ATTTTCTACG ACTTCTAGTC AACCCACGTG CTCACCCAAT GTAGCTTGAC CTAGAGTTGT CGCCATTCTA GGAACTCTCA AAAGCGGGGC

                                                                                  scrFI
                                                                                  nciI
                                                        aciI                      mspI
                                                       thaI                       hpaII
                                                       fnuDII/mvnI                dsaV
        maeII                 hgiAI/aspHI              bstUI                      cauII
       psp1406I               bsp1286  tru9I           bsh1236I                hinlI/acyI
      xmnI                    bsiHKAI  mseI           hinPI                      hgaI                                  aciI
      asp700                  bmyI    ahaIII/draI     hhaI/cfoI                  ahaII/bsaHI    bcgI   mcrI  fnu4HI
4701  AAGAACGTTT TCCAATGATG AGCACTTTTA AAGTTCTGCT ATGTGGCGCG GTATTATCCC GTGATGACGC CGGGCAAGAG CAACTCGGTC GCCGCATACA
      TTCTTGCAAA AGGTTACTAC TCGTGAAAAT TTCAAGACGA TACACCGCGC CATAATAGGG CACTACTGCG GCCCGTTCTC GTTGAGCCAG CGGCGTATGT

                                  rsaI
                                  csp6I   bsrI                                                          fnu4HI
         ddeI                    scaI hphI   maeIII      sfaNI     fokI   nlaIII                       bbvI        nlaIII
4801  CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA GAAAAGCATC TTACGGATGG CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG
      GATAAGAGTC TTACTGAACC AACTCATGAG TGGTCAGTGT CTTTTCGTAG AATGCCTACC GTACTGTCAT TCTCTTAATA CGTCACGACG GTATTGGTAC

                                                  sau96I
                                                  avaII                                                    maeIII
                                          sau3AI  asuI                                                   nlaIII
                    haeIII/palI           mboI/ndeII[dam-]                                             sau3AI
                   eaeI                   dpnI[dam+]                                                   mboI/ndeII[dam-]   sau3AI
                   cfrI                   dpnII[dam-]                                                  dpnI[dam+]         mboI/ndeII[dam-]
                  fnu4HI                 pvuI/bspCI                                                    dpnII[dam-]        dpnI[dam+]
                  aciI                    mcrI   mnlI          aluI  aciI              nlaIII alwI[dam-]                 dpnII[dam-]
4901  AGTGATAACA CTGCGGCCAA CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC GCTTTTTTGC ACAACATGGG GGATCATGTA ACTCGCCTTG
      TCACTATTGT GACGCCGGTT GAATGAAGAC TGTTGCTAGC CTCCTGGCTT CCTCGATTGG CGAAAAAACG TGTTGTACCC CCTAGTACAT TGAGCGGAAC
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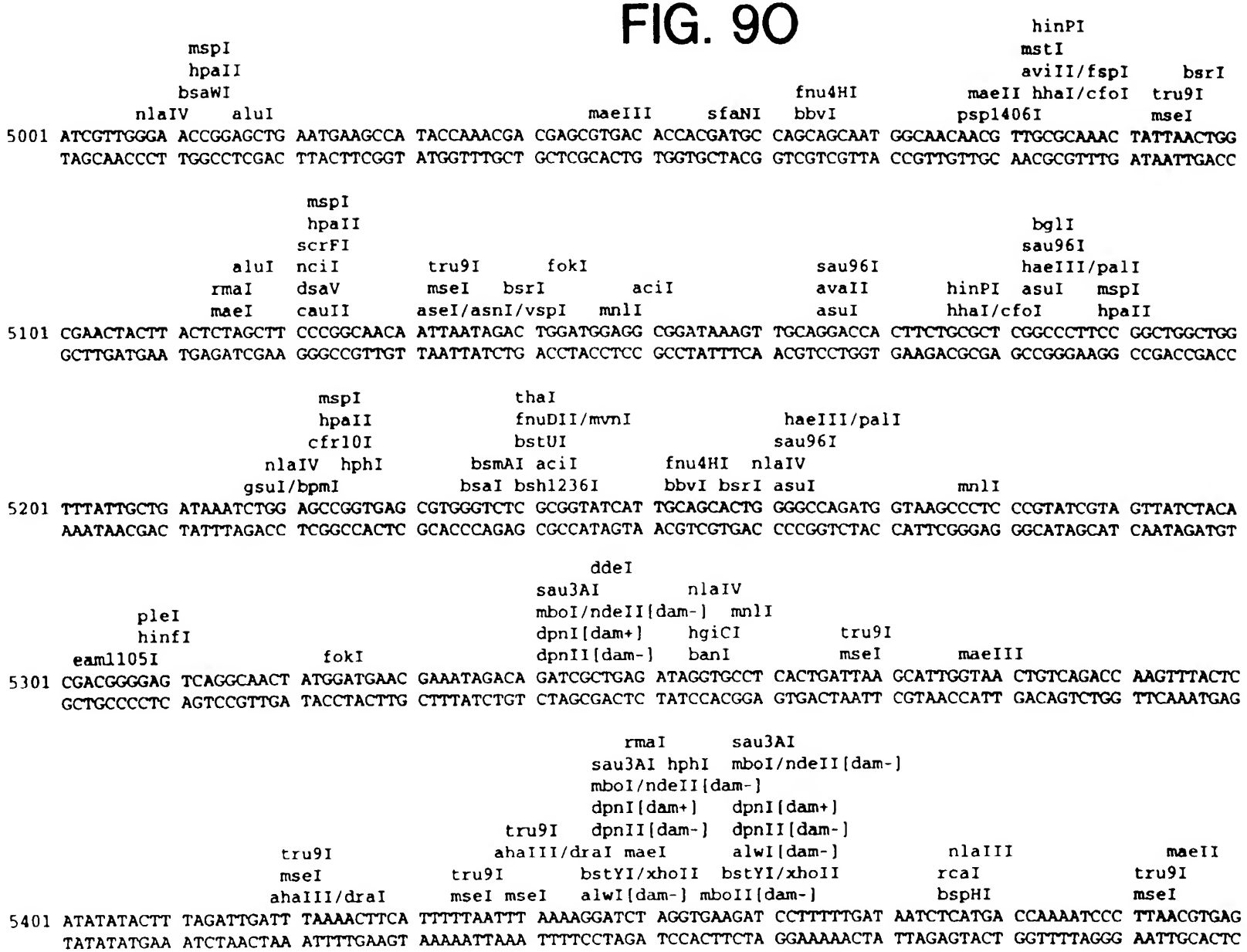
FIG. 90



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BNSDOCID <WO__9604391A1_I_>

SUBSTITUTE SHEET (RULE 26)

FIG. 9P

```
                                                                                                    sau3AI
                                                                                                    mboI/ndeII[dam-]
                                                                                                    dpnI[dam+]  sau3AI          thaI
                                                                                                    dpnII[dam-] mboI/ndeII[dam-]
                                                                                                   bstYI/xhoII  dpnI[dam+]      fnuDII/mvnI
                                                                                  sau3AI  alwI[dam-]   dpnII[dam-]     bstUI
                                                                                  mboI/ndeII[dam-]     alwI[dam-]      bsh1236I
                                                  hgaI                            dpnI[dam+] mboII[dam-]              hinPI       fnu4HI
                                               ddeI                               dpnII[dam-]         bstYI/xhoII     hhaI/cfoI   bbvI
5501 TTTTCGTTCC ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTTTCTGC GCGTAATCTG CTGCTTGCAA ACAAAAAAAC
     AAAAGCAAGG TGACTCGCAG TCTGGGGCAT CTTTTCTAGT TTCCTAGAAG AACTCTAGGA AAAAAAGACG CGCATTAGAC GACGAACGTT TGTTTTTTTG

                                        sau3AI
                                        mboI/ndeII[dam-]
                                        dpnI[dam+]
                                        dpnII[dam-]
                                       alwI[dam-]
                        aciI            mspI                                                bsrI            hinPI
          aciI   nspBII                 hpaII      aluI                                  maeIII   eco57I     hhaI/cfoI
5601 CACCGCTACC AGCGGTGGTT TGTTTGCCGG ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA ATACTGTCCT
     GTGGCGATGG TCGCCACCAA ACAAACGGCC TAGTTCTCGA TGGTTGAGAA AAAGGCTTCC ATTGACCGAA GTCGTCTCGC GTCTATGGTT TATGACAGGA

                                                                                                                fnu4HI
                                                                                                          alwNI   bbvI
          rmaI                  haeIII/palI                                                           bsrI    fnu4HI
          maeI      bslI    haeI                        scfI     aciI        mnlI                  maeIII     bbvI    bsrI
5701 TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC
     AGATCACATC GGCATCAATC CGGTGGTGAA GTTCTTGAGA CATCGTGGCG GATGTATGGA GCGAGACGAT TAGGACAATG GTCACCGACG ACGGTCACCG

                         scrFI                                      aciI                        hgiAI/aspHI
                         nciI                                     nspBII                        bsp1286
                         mspI                          mspI       fnu4HI                        bsiHKAI
                         hpaII                         hpaII      bbvI                          bmyI
                         dsaV     pleI                 bsaWI    hinPI mcrI                      apaLI/snoI
                         cauII    hinfI          maeIII         hhaI/cfoI                       alw44I/snoI   aluI
5801 GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA CGGGGGGTTC GTGCACACAG CCCAGCTTGG
     CTATTCAGCA CAGAATGGCC CAACCTGAGT TCTGCTATCA ATGGCCTATT CCGCGTCGCC AGCCCGACTT GCCCCCCAAG CACGTGTGTC GGGTCGAACC
```

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FIG. 9Q

```
                                                                                                                    mspI
                                                                                  hinPI                             hpaII
                                                                                  hhaI/cfoI                         bslI     fnu4HI
                                dde I      scf I                                  hae II                  aci I      bsaWI    aci I
5901 AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA GCATTGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG
     TCGCTTGCTG GATGTGGCTT GACTCTATGG ATGTCGCACT CGTAACTCTT TCGCGGTGCG AAGGGCTTCC CTCTTTCCGC CTGTCCATAG GCCATTCGCC

                                                scrFI
                                                mvaI          scrFI
                                                ecoRII  mvaI
                                                dsaV    ecoRII
                                                bstNI         dsaV
                                hinPI  mnlI     bsaJI         bstNI                                                taqI
                                hhaI/cfoI  aluI apyI[dcm+]    apyI[dcm+]                          mnlI drdI    hgaI
6001 CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA
     GTCCCAGCCT TGTCCTCTCG CGTGCTCCCT CGAAGGTCCC CCTTTGCGGA CCATAGAAAT ATCAGGACAG CCCAAAGCGG TGGAGACTGA ACTCGCAGCT

                                                                                                haeIII/palI
                                                                    haeIII/palI     scrFI
                                                                    fnu4HI          mvaI
                                                                    aciI            ecoRII
                                                                    thaI bslI       dsaV                    nlaIII
                                                                    fnuDII/mvnI     bstNI bslI                  nspI
                                        nlaIV                       bstUI           apyI[dcm+]    haeIII/palI   nspHI
                    sfaNI               aciI                        bsh1236I    nlaIV  haeI       haeI        afl III
6101 TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT
     AAAAACACTA CGAGCAGTCC CCCCGCCTCG GATACCTTTT TGCGGTCGTT GCGCCGGAAA AATGCCAAGG ACCGGAAAAC GACCGGAAAA CGAGTGTACA

                                                                                    fnu4HI              fnu4HI
                                                                                    bbvI                bbvI pleI
                                tfiI                                        bsrBI   aciI                hinPI   hinfI
                                hinfI                       aciI     aluI   aciI  fnu4HI      mcrI      hhaI/cfoI
6201 TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC
     AGAAAGGACG CAATAGGGGA CTAAGACACC TATTGGCATA ATGGCGGAAA CTCACTCGAC TATGGCGAGC GGCGTCGGCT TGCTGGCTCG CGTCGCTCAG
```

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FIG. 9R

```
                                                       thaI
                                                       fnuDII/mvnI
                                                       bstUI
                                                       bsh1236I
                                                     hinPI
                                                     hhaI/cfoI
                                                    thaI
                                                    fnuDII/mvnI
             sapI  hinPI                            bstUI                tru9I   aluI
             mboII hhaI/cfoI                        bsh1236I haeIII/palI        pvuII
             earI/ksp632I                 mnlI     bslI      eaeI  tfiI aseI/asnI/vspI
       mnlI    aciI     haeII           aciI       aciI      cfrI  hinfI mseI   nspBII          bsrI
6301 AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG CCTCTCCCCG CGCGTTGGCC GATTCATTAA TCCAGCTGGC ACGACAGGTT TCCCGACTGG
     TCACTCGCTC CTTCGCCTTC TCGCGGGTTA TGCGTTTGGC GGAGAGGGGC GCGCAACCGG CTAAGTAATT AGGTCGACCG TGCTGTCCAA AGGGCTGACC

                                                             scrFI
                                                             mvaI
                                                             ecoRII
                                                             dsaV
                               tru9I                   nlaIV bstNI
                 hinPI         mseI     maeIII         hgiCI apyI[dcm+]             mspI
     aciI        hhaI/cfoI   aseI/asnI/vspI mnlI       banI bsaJI                   hpaII
6401 AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTACC TCACTCATTA GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA
     TTTCGCCCGT CACTCGCGTT GCGTTAATTA CACTCAATGG AGTGAGTAAT CCGTGGGGTC CGAAATGTGA AATACGAAGG CCGAGCATAC AACACACCTT

                                                          tru9I
                                                          mseI
                                                         aseI/asnI/vspI
       aciI                                              xmnI
      bsrBI                         aluI       nlaIII    asp700
6501 TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC GAATTAA
     AACACTCGCC TATTGTTAAA GTGTGTCCTT TGTCGATACT GGTACTAATG CTTAATT

>length: 6557
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FIG. 10A

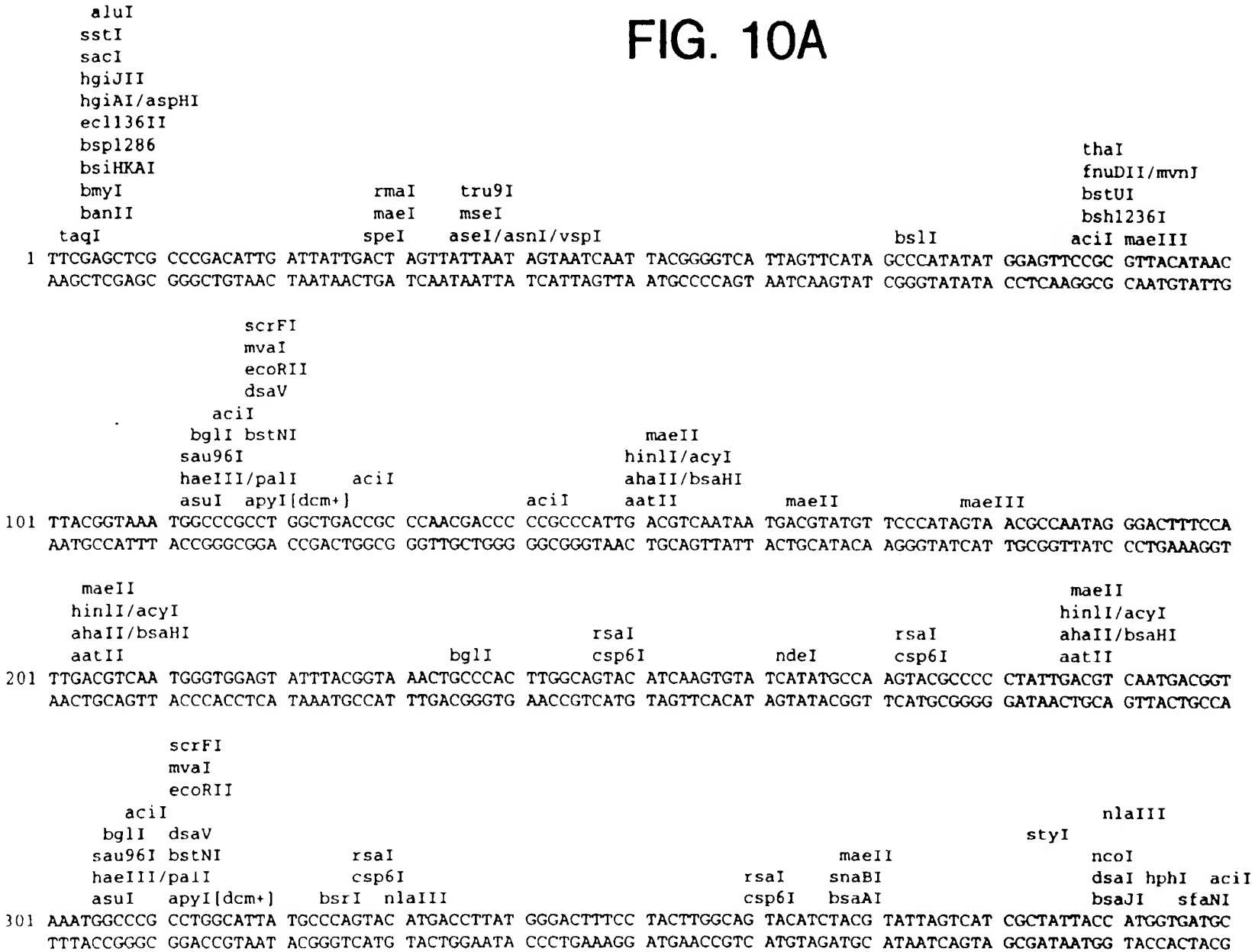

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FIG. 10B

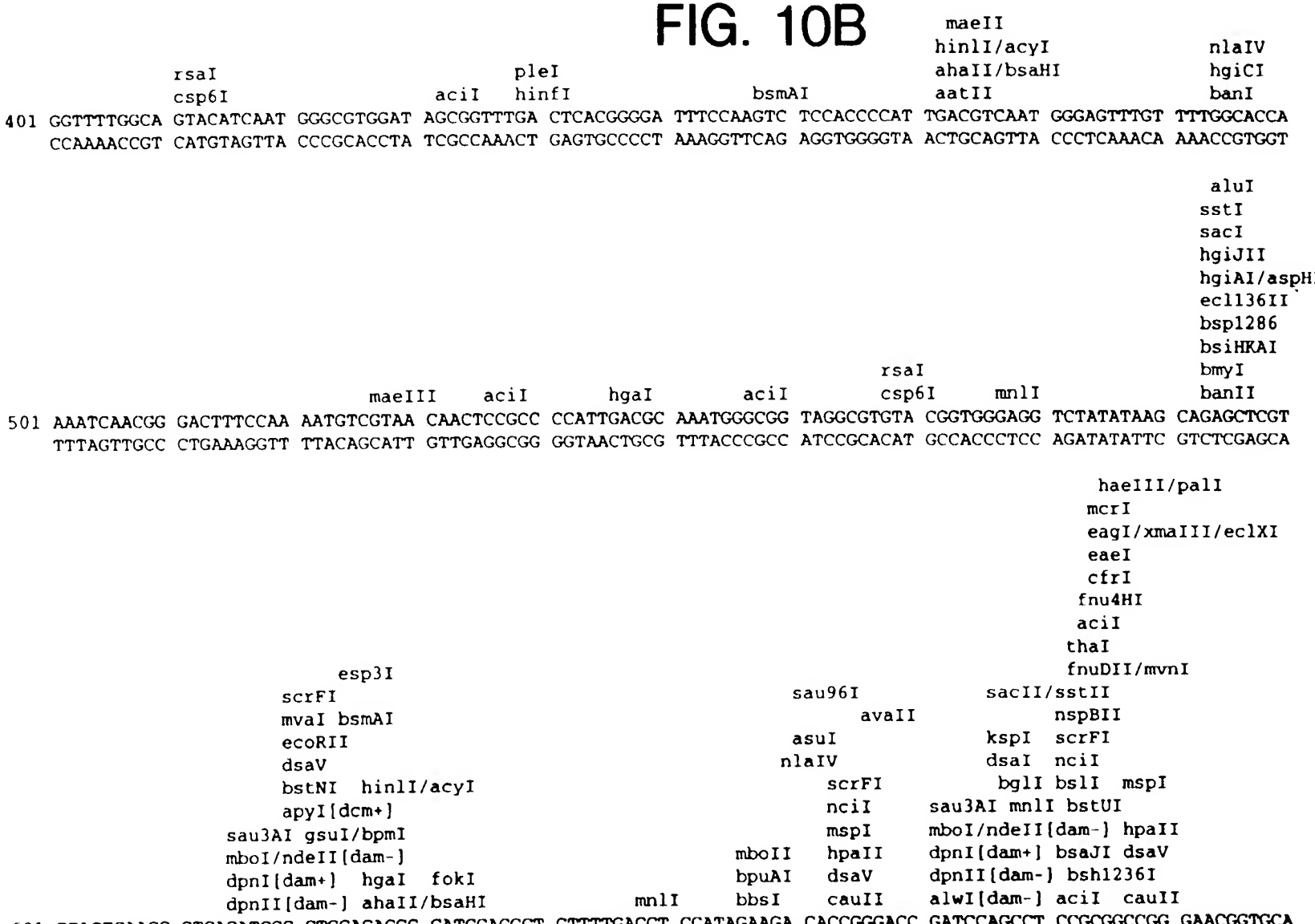

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FIG. 10C

```
                tfiI                                                                                                  fnu4HI
             aciI                                                                                                     aciI
            thaI hinfI                                                                                  bstXI        thaI
            fnuDII/mvnI                                  aciI                                  sau96I    styI       fnuDII/mvnI tru9I
            bstUI                                  maeII rsaI          pleI scfI haeIII/palI                        bstUI       mseI
            bsh1236I                          maeIII    csp6I   scfI hinfI     asuI      bsaJI                    bsh1236I    aseI/asnI/vspI
701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA
    AACCTTGCGC CTAAGGGGCA CGGTTCTCAC TGCATTCATG GCGGATATCT CAGATATCCG GGTGGGGGAA CCGAAGCAAT CTTGCGCCGA TGTTAATTAT

                                                                                                                  sau96I
                                                                                                                  avaII
                                                                                                                  asuI
                                                                                                               scrFI
                                                                                                               mvaI
                                                                                                               ecoRII
                                                                                                               dsaV
                                                                                                               bstNI
                                                                                                               apyI[dcm+]
                                          maeIII                                                                  bslI bsaJI
                                         hphI   scfI          fokI
801 CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC
    GTATTGGAAT ACATAGTATG TGTATGCTAA ATCCACTGTG ATATCTTATT GTAGGTGAAA CGGAAAGAGA GGTGTCCACA GGTGAGGGTC CAGGTTGACG

                                                  hinlI/acyI
                                                  ahaII/bsaHI
                                                  aatII
                                               thaI                        taqI[dam-]
                                               fnuDII/mvnI                 sau3AI              bsmAI
                                               bstUI                       mboI/ndeII[dam-]    esp3I
                    aluI  taqI                 aciI maeII                  dpnI[dam+]          tth111I/aspI
     mnlI       hindIII                        hphI bsh1236I    taqI       dpnII[dam-] taqI     hgaI          sfaNI
     bsaJI   ddeI     claI/bsp106
901 ACCTCGGTTC TAAGCTTATC GATATGAAAA AGCCTGAACT CACCGCGACG TCTGTCGAGA AGTTTCTGAT CGAAAAGTTC GACAGCGTCT CCGACCTGAT
    TGGAGCCAAG ATTCGAATAG CTATACTTTT TCGGACTTGA GTGGCGCTGC AGACAGCTCT TCAAAGACTA GCTTTTCAAG CTGTCGCAGA GGCTGGACTA

                                                                                                        hinPI
                                                                                                     fnu4HI
      aluI              tfiI                                                                         bbvI
     fnu4HI             hinfI                  taqI
     bbvI       mnlI  mboII                  aluI          mnlI                    aciI       aluI hhaI/cfoI
1001 GCAGCTCTCG GAGGGCGAAG AATCTCGTGC TTTCAGCTTC GATGTAGGAG GGCGTGGATA TGTCCTGCGG GTAAATAGCT GCGCCGATGG TTTCTACAAA
     CGTCGAGAGC CTCCCGCTTC TTAGAGCACG AAAGTCGAAG CTACATCCTC CCGCACCTAT ACAGGACGCC CATTTATCGA CGCGGCTACC AAAGATGTTT
```

FIG. 10D

```
                                     hinPI
                                     hhaI/cfoI    mspI
                                    thaI          hpaII
                                   aciI          mroI
                                haeIII/palI      bspMII
                               mcrI fnuDII/mvnI  bspEI
      sau3AI                   eagI/xmaIII/eclXI bsaWI
      mboI/ndeII(dam-)         eaeI bstUI     tfiI
      dpnI(dam+)               cfrI bsh1236I  hinfI                  ecoRI
      dpnII(dam-)          sfaNI  fnu4HI  bslI    accIII             apoI                       sfaNI  aciI
1101  GATCGTTATG TTTATCGGCA CTTTGCATCG GCCGCGCTCC CGATTCCGGA AGTGCTTGAC ATTGGGGAAT TCAGCGAGAG CCTGACCTAT TGCATCTCCC
      CTAGCAATAC AAATAGCCGT GAAACGTAGC CGGCGCGAGG GCTAAGGCCT TCACGAACTG TAACCCCTTA AGTCGCTCTC GGACTGGATA ACGTAGAGGG

                                                                                                 mcrI
                                                                                                 eagI/xmaIII/eclXI
                                                                                                 eaeI
                                                                          styI                  fnu4HI
        hgiAI/aspHI                                                 thaI    ncoI       sau3AI   aciI
        bsp1286                                                     fnuDII/mvnI        mboI/ndeII(dam-)
        bsiHKAI                                             fnu4HI  bstUI   dsaI       dpnI(dam+)   sau3AI
        bmyI                                                bbvI  mcrI    haeIII/palI  dpnII(dam-)  mboI/ndeII(dam-)
        apaLI/snoI                                         scfI   mspI bsh1236I bsaJI sfaNI  fnu4HI   dpnI(dam+)
        alw44I/snoI    maeII                       nspBII  pstI   hpaII    mnlI nlaIII  pvuI/bspCI haeIII/palI
       bslI draIII   maeIII       bspMI            aciI    bsgI  cfr10I aciI haeI  fokI  mcrI bbvI cfrI dpnII(dam-)
1201  GCCGTGCACA GGGTGTCACG TTGCAACACC TGCCTGAAAC CGAACTGCCC GCTGTTCTGC AGCCGGTCGC GGAGGCCATG GATGCGATCG CTGCGGCCGA
      CGGCACGTGT CCCACAGTGC AACGTTGTGG ACGGACTTTG GCTTGACGGG CGACAAGACG TCGGCCAGCG CCTCCGGTAC CTACGCTAGC GACGCCGGCT

                                   sau96I                                                 thaI      sau3AI
                                   avaII                                                  fnuDII/mvnI    nlaIII
                                   asuI                                                   bstUI     mboI/ndeII(dam-)
                           sau96I  rsrII/cspI                                             bsh1236I  dpnI(dam+)
                 aciI      haeIII/palI aciI   tfiI                                       hinPI      dpnII(dam-)
       ddeI     bsrBI      asuI    cpoI       hinfI              nlaIII           ndeI hhaI/cfoI  alwI(dam-)
1301  TCTTAGCCAG ACGAGCGGGT TCGGCCCATT CGGACCGCAA GGAATCGGTC AATACACTAC ATGGCGTGAT TTCATATGCG CGATTGCTGA TCCCCATGTG
      AGAATCGGTC TGCTCGCCCA AGCCGGGTAA GCCTGGCGTT CCTTAGCCAG TTATGTGATG TACCGCACTA AAGTATACGC GCTAACGACT AGGGGTACAC

                                             hinPI                                                        draIII
                                             hhaI/cfoI                                                    nlaIV
                                            thaI                                     bsaJI                hgiCI
                                            fnuDII/mvnI                            haeIII/palI            banI
                             tth111I/aspI   bstUI                      sfaNI       sau96I               mspI
         bsrI           drdI          hgaI  bsh1236I    taqI    aluI              asuI mnlI        bslI  hpaII
1401  TATCACTGGC AAACTGTGAT GGACGACACC GTCAGTGCGT CCGTCGCGCA GGCTCTCGAT GAGCTGATGC TTTGGGCCGA GGACTGCCCC GAAGTCCGGC
      ATAGTGACCG TTTGACACTA CCTGCTGTGG CAGTCACGCA GGCAGCGCGT CCGAGAGCTA CTCGACTACG AAACCCGGCT CCTGACGGGG CTTCAGGCCG
```


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FIG. 10E

```
                aciI
               thaI
               fnuDII/mvnI
          hgiAI/aspHI
          bsp1286
          bsiHKAI                                          aciI
          bmyI  bstUI                                     fnu4HI
          apaLI/snoI                                     haeIII/palI
          alw44I/snoI                                   eaeI          aciI         gsuI/bpmI              tfiI bslI
     mnlI      bsh1236I   nlaIV                         cfrI        nspBII       bsrI     mnlI            hinfI
1501 ACCTCGTGCA CGCGGATTTC GGCTCCAACA ATGTCCTGAC GGACAATGGC CGCATAACAG CGGTCATTGA CTGGAGCGAG GCGATGTTCG GGGATTCCCA
     TGGAGCACGT GCGCCTAAAG CCGAGGTTGT TACAGGACTG CCTGTTACCG GCGTATTGTC GCCAGTAACT GACCTCGCTC CGCTACAAGC CCCTAAGGGT

                                                                                                        fnu4HI
                                                                                                       thaI
                                                                                                       fnuDII/mvnI
                                                                                                       bstUI
                                                                                                       bsh1236I
                                                                                                      sacII/sstII
                                                                                                      nspBII
                                                                                                      kspI
                                                                                          mspI        dsaI
                                                                                          hpaII       bsaJI
                                                                                         mroI         aciI
                                                                                         bspMII      fnu4HI
                                                                                         bspEI       sau3AI aciI
                                                                                         bsaWI       mboI/ndeII[dam-]
                                        dsaI                                                         dpnI[dam+]
                                   haeIII/palI                                 rsaI    aciI   fokI  dpnII[dam-]
                              mboII  mnlI bsaJI                    fnu4HI     csp6I  bsrBI   sfaNI   aluI   alwI[dam-]
        mnlI          mboII   gsuI/bpmI                           bbvI   maeII   taqI    mnlI  accIII
1601 ATACGAGGTC GCCAACATCT TCTTCTGGAG GCCGTGGTTG GCTTGTATGG AGCAGCAGAC GTACTTCGAG CGGAGGCATC CGGAGCTTGC AGGATCGCCG
     TATGCTCCAG CGGTTGTAGA AGAAGACCTC CGGCACCAAC CGAACATACC TCGTCGTCTG CATGAAGCTC GCCTCCGTAG GCCTCGAACG TCCTAGCGGC

          scrFI
          nciI
          mspI
          hpaII
          dsaV                                                                    aluI
          cauII                                                                  fnu4HI                      hgaI
                                                                                 bbvI      hinPI    taqI   drdI
     nlaIV                aciI                                  aluI   nlncII/hindII taqI  sfaNI       hhaI/cfoI  sfaNI
1701 CGGCTCCGGG CGTATATGCT CCGCATTGGT CTTGACCAAC TCTATCAGAG CTTGGTTGAC GGCAATTTCG ATGATGCAGC TTGGGCGCAG GGTCGATGCG
     GCCGAGGCCC GCATATACGA GGCGTAACCA GAACTGGTTG AGATAGTCTC GAACCAACTG CCGTTAAAGC TACTACGTCG AACCCGCGTC CCAGCTACGC
```

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FIG. 10F

```
          nlaIV                                                       haeIII/palI
         mspI                                                        mcrI
         hpaII  scrFI                                                eagI/xmaIII/eclXI
         bslI   nciI                                                 eaeI
        mroI    mspI                                                 cfrI
        bspMII  hpaII                                               fnu4HI
        bspEI[dam-]                                                 aciI
        bsaWI   dsaV                                               thaI
        accIII[dam-]                                               fnuDII/mvnI
       sau3AI   cauII                                              bstUI
       mboI/ndeII[dam-]                                            bsh1236I  sau96I              rsaI
       dpnI[dam+]                                                            
       dpnII[dam-]                rsaI                            hinPI     avaII                csp6I
       alwI[dam-]                 csp6I          aciI             hhaI/cfoI asuI                scaI
1801 ACGCAATCGT CCGATCCGGA GCCGGGACTG TCGGGCGTAC ACAAATCGCC CGCAGAAGCG CGGCCGTCTG GACCGATGGC TGTGTAGAAG TACTCGCCGA
     TGCGTTAGCA GGCTAGGCCT CGGCCCTGAC AGCCCGCATG TGTTTAGCGG GCGTCTTCGC GCCGGCAGAC CTGGCTACCG ACACATCTTC ATGAGCGGCT

                                                                            scrFI
                                                                            nciI
                                                                            mspI
                                                                            hpaII
                                                                            dsaV
                                                                           xmaI/pspAI
                                                                           smaI
                                                                           scrFI
                                                                           nciI
                                                                           dsaV
                                                                           cauII
                                                                           bsaJI
                                                                           avaI
                                                                          bsaJI
                                                                        sau3AI                          sau96I
                                                                        mboI/ndeII[dam-]       aciI     haeIII/palI
                                                                        dpnI[dam+]            fnu4HI    asuI
                                                                        dpnII[dam-]           bglI  nlaIII
                                                                        alwI[dam-]           sfiI  styI
                                                                       nlaIV cauII          eaeI  ncoI
       hinlI/acyI                                             mcrI     bstYI/xhoII          cfrI  dsaI
       hgaI                          mnlI                    bslI      bamHI bsaJI ecoRI  taqI haeIII/palI
       ahaII/bsaHI                 bsaJI                  sfaNI        alwI[dam-]  apoI  claI/bsp106 bsaJI
1901 TAGTGGAAAC CGACGCCCCA GCACTCGTCC GAGGGCAAAG GAATAGAGTA GATGCCGACC GAAGGATCCC CGGGGAATTC AATCGATGGC CGCCATGGCC
     ATCACCTTTG GCTGCGGGGT CGTGAGCAGG CTCCCGTTTC CTTATCTCAT CTACGGCTGG CTTCCTAGGG GCCCCTTAAG TTAGCTACCG GCGGTACCGG
```


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FIG. 10G

```
                    aluI                                                                                    rmaI
                   fnu4HI                                                                                   
                   bbvI          maeIII              sfaNI   apoI                                      bsmI maeI
2001 CAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA CAAATTTCAC AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG
     GTTGAACAAA TAACGTCGAA TATTACCAAT GTTTATTTCG TTATCGTAGT GTTTAAAGTG TTTATTTCGT AAAAAAAGTG ACGTAAGATC AACACCAAAC

                                        sau3AI
                                        mboI/ndeII[dam-]
                                        dpnI[dam+]
                                        dpnII[dam-]
                                       pvuI/bspCI
                                       mcrI
                                      taqI[dam-]  tru9I                haeIII/palI
                                     claI/bsp106[dam-]                 haeI
                                    sau3AI        mseI       fnu4HI styI
                                    mboI/ndeII[dam-]         bbvI   ncoI
                                    dpnI[dam+] xmnI        hinPI    dsaI
                                    dpnII[dam-]  aseI/asnI/vspI     bsaJI
                              nlaIII  alwI[dam-]  asp700      hhaI/cfoI nlaIII          mnlI      mnlI
2101 TCCAAACTCA TCAATGTATC TTATCATGTC TGGATCGATC GGGAATTAAT TCGGCGCAGC ACCATGGCCT GAAATAACCT CTGAAAGAGG AACTTGGTTA
     AGGTTTGAGT AGTTACATAG AATAGTACAG ACCTAGCTAG CCCTTAATTA AGCCGCGTCG TGGTACCGGA CTTTATTGGA GACTTTCTCC TTGAACCAAT

      rsaI                                                                  nlaIV
      csp6I                                                                scrFI                          sfaNI
     nlaIV                                                                 mvaI                              ppu10I
     kpnI                                                                  ecoRII                        nsiI/avaIII
     hgiCI                                                                 dsaV                         nlaIII
     banI                          aluI                                    bstNI                             sphI
     asp718     mnlI               pvuII                                   apyI[dcm+]                        nspI
     acc65I  ddeI  aciI            nspBII                                 bsaJI                             nspHI
2201 GGTACCTTCT GAGGCGGAAA GAACCAGCTG TGGAATGTGT GTCAGTTAGG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC
     CCATGGAAGA CTCCGCCTTT CTTGGTCGAC ACCTTACACA CAGTCAATCC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG

                                        nlaIV
                          scrFI        scrFI
                          mvaI              mvaI                              ppu10I
                          ecoRII       ecoRII                       nsiI/avaIII
                          dsaV         dsaV                        nlaIII
                          bstNI             bstNI                          sphI
                          apyI[dcm+]        apyI[dcm+]                     nspI sfaNI
                         sexAI             bsaJI                           nspHI                              aciI
2301 ATCTCAATTA GTCAGCAACC AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA CCATAGTCCC
     TAGAGTTAAT CAGTCGTTGG TCCACACCTT TCAGGGGTCC GAGGGGTCGT CCGTCTTCAT ACGTTTCGTA CGTAGAGTTA ATCAGTCGTT GGTATCAGGG
```

FIG. 10H

```
                                                                                                  fnu4HI
                                                                                                  bglI
                                                           nlaIII                                sfiI
                                                           styI                                  haeIII/palI
                                                           ncoI                                mnlI
                         aciI             bsrI          bslI dsaI                            haeIII/palI
                   aciI fokI           aciI      aciI   aciI bsaJI                          mnlI bsaJI aciI
2401 GCCCCTAACT CCGCCCATCC CGCCCCTAAC TCCGCCCAGT TCCGCCCATT CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC
     CGGGGATTGA GGCGGGTAGG GCGGGGATTG AGGCGGGTCA AGGCGGGTAA GAGGCGGGGT ACCGACTGAT TAA AAAAT AAATACGTCT CCGGCTCCGG

                                                                                         scrFI
                                                                                         nciI
                                                                                         mspI
                                                                                         hpaII
                                                                                         dsaV
                                                         styI                          haeIII/palI
                                                         bsaJI                       mcrI
                                                         blnI                        eagI/xmaIII/eclXI
                                                      haeIII/palI          rmaI      eaeI
        haeIII/palI                                  stuI rmaI             maeI      cfrI
   mnlI      ddeI                      mnlI          haeI maeI            nheI      mspI cauII
   bsaJI mnlI   aluI               mnlI            mnlI avrII           aluI aluI  hpaII
2501 GCCTCGGCCT CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTTGGAGG CCTAGGCTTT TGCAAAAAGC TAGCTTATCC GGCCGGGAAC GGTGCATTGG
     CGGAGCCGGA GACTCGATAA GGTCTTCATC ACTCCTCCGA AAAAACCTCC GGATCCGAAA ACGTTTTTCG ATCGAATAGG CCGGCCCTTG CCACGTAACC

        tfiI
        hinfI                                                                            fnu4HI
     aciI                                                                                aciI
    thaI                                                    bstXI                      thaI
    fnuDII/mvnI                          aciI         scfI sau96I    styI              fnuDII/mvnI   tru9I
    bstUI                   pleI      rsaI        pleI    haeIII/palI                  bstUI         mseI
    bsh1236I                hinfI     csp6I  scfI hinfI    asuI       bsaJI            bsh1236I   aseI/asnI/vspI
2601 AACGCGGATT CCCCGTGCCA AGAGTCAGGT AAGTACCGCC TATAGAGTCT ATAGGCCCAC CCCCTTGGCT TCGTTAGAAC GCGGCTACAA TTAATACATA
     TTGCGCCTAA GGGGCACGGT TCTCAGTCCA TTCATGGCGG ATATCTCAGA TATCCGGGTG GGGGAACCGA AGCAATCTTG CGCCGATGTT AATTATGTAT
```


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FIG. 10I

```
                sau3AI
                mboI/ndeII[dam-]
                dpnI[dam+]                                                    sau96I
                dpnII[dam-]                                                   avaII
                alwI[dam-]                                                    asuI
              taqI[dam-]                                                    scrFI
             claI/bsp106[dam-]                                              mvaI                                  rmaI
            sau3AI                                                          ecoRII              thaI    maeI
            mboI/ndeII[dam-]                                                dsaV                fnuDII/mvnI
            dpnI[dam+]                                                      bstNI                     bstUI  nheI
            dpnII[dam-]                                                     apyI[dcm+]        mnlI    bsh1236I  aluI
           alwI[dam-]              fokI                              bslI  bsaJI             bsaJI  nruI   aluI
2701 ACCTTTTGGA TCGATCCTAC TGACACTGAC ATCCACTTTT TCTTTTTCTC CACAGGTGTC CACTCCCAGG TCCAACTGCA CCTCGGTTCG CGAAGCTAGC
     TGGAAAACCT AGCTAGGATG ACTGTGACTG TAGGTGAAAA AGAAAAAGAG GTGTCCACAG GTGAGGGTCC AGGTTGACGT GGAGCCAAGC GCTTCGATCG

                            nlaIII
                           styI
                           pflMI
                           ncoI
        sfaNI     ecoRI    dsaI                                                  rsaI              aluI
     fnu4HI taqI  apoI     bslI fokI                          rmaI            gsuI/bpmI             pvuII tth111I/aspI
     bbvI claI/bsp106      bsaJI        nlaIII  fokI        maeI            bsrI   csp6I     ecoRV nspBII   bsrI
2801 TTGGGCTGCA TCGATTGAAT TCCACCATGG GATGGTCATG TATCATCCTT TTTCTAGTAG CAACTGCAAC TGGAGTACAT TCAGATATCC AGCTGACCCA
     AACCCGACGT AGCTAACTTA AGGTGGTACC CTACCAGTAC ATAGTAGGAA AAAGATCATC GTTGACGTTG ACCTCATGTA AGTCTATAGG TCGACTGGGT

          aluI
         sstI
         sacI
         hgiJII
         hgiAI/aspHI
         ecl136II
         bsp1286
         bsiHKAI
         bmyI                              hphI
         banII          mnlI               maeIII    bspMI                taqI                          bsrI
    avaI          aciI                    bstEII   hphI        bsrI      hgaI          hphI   aluI  nlaIII
2901 GTCCCCGAGC TCCCTGTCCG CCTCTGTGGG CGATAGGGTC ACCATCACCT GCCGTGCCAG TCAGAGCGTC GATTACGATG GTGATAGCTA CATGAACTGG
     CAGGGGCTCG AGGGACAGGC GGAGACACCC GCTATCCCAG TGGTAGTGGA CGGCACGGTC AGTCTCGCAG CTAATGCTAC CACTATCGAT GTACTTGACC
```


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FIG. 10J

```
                                                                                                           mspI
                                                                                                           hpaII
                                                                                                           bslI
                                                                                                          bsaWI
                                                            gsuI/bpmI                              sau3AI
                                                           scrFI                                        mboI/ndeII[dam-]
                                                           mvaI                                         dpnI[dam+]
                                                   haeIII/palI                                dpnII[dam-]
                  scrFI                      fnu4HI       ecoRII                                    alwI[dam-]
                  mvaI                           aciI          dsaV                                nlaIV
                  ecoRII                     thaI mnlI    bstNI                                    bstYI/xhoII
                  dsaV                       fnuDII/mvnI  apyI[dcm+]  pleI                              bamHI
                  bstNI    aluI                  bstUI      rsaI    pleI gsuI/bpmI                      alwI[dam-]
                  apyI[dcm+]                     bsh1236I   csp6I   hinfI   hinfI
3001 TATCAACAGA AACCAGGAAA AGCTCCGAAA CTACTGATTT ACGCGGCCTC GTACCTGGAG TCTGGAGTCC CTTCTCGCTT CTCTGGATCC GGTTCTGGGA
     ATAGTTGTCT TTGGTCCTTT TCGAGGCTTT GATGACTAAA TGCGCCGGAG CATGGACCTC AGACCTCAGG GAAGAGCGAA GAGACCTAGG CCAAGACCCT

                                                                                            sau3AI                   styI
                                                                                            mboI/ndeII[dam-]          bsaJI
                                                                                            dpnI[dam+]             rsaI
                                                                                            dpnII[dam-]            csp6I
                                                                                            alwI[dam-]            nlaIV
                                          fnu4HI  mboII                                    nlaIV                  kpnI
                                          bbvI    bpuAI                                    bstYI/xhoII            hgiCI
                                         scfI     bbsI                                     bamHI rsaI             banI
                                         pstI   mspI                                     mnlI    csp6I            asp718
                                         bsgI   hpaII                              maeIII  alwI[dam-]             acc65I
3101 CGGATTTCAC TCTGACCATC AGCAGTCTGC AGCCGGAAGA CTTCGCAACT TATTACTGTC AGCAAAGTCA CGAGGATCCG TACACATTTG GACAGGGTAC
     GCCTAAAGTG AGACTGGTAG TCGTCAGACG TCGGCCTTCT GAAGCGTTGA ATAATGACAG TCGTTTCAGT GCTCCTAGGC ATGTGTAAAC CTGTCCCATG

               sau3AI
               mboI/ndeII[dam-]                 mboII
               dpnI[dam+]            fnu4HI     bpuAI       aciI                                      mnlI
               dpnII[dam-]           bbvI       bbsI    mboII
3201 CAAGGTGGAG ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG
     GTTCCACCTC TAGTTTGCTT GACACCGACG TGGTAGACAG AAGTAGAAGG GCGGTAGACT ACTCGTCAAC TTTAGACCTT GACGGAGACA ACACACGGAC

                                                                                       scrFI
                                                                                       mvaI
                                                                                       ecoRII
                                                                                       dsaV
                                                                                       bstNI
                                    haeIII/palI                                        apyI[dcm+]
       xmnI                        haeI      rsaI                mnlI
       asp700                     mnlI      csp6I                bslI        maeIII  bsaJI          maeIII
3301 CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA
     GACTTATTGA AGATAGGGTC TCTCCGGTTT CATGTCACCT TCCACCTATT GCGGGAGGTT AGCCCATTGA GGGTCCTCTC ACAGTGTCTC GTCCTGTCGT
```


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FIG. 10K

```
                                                                                                               sstI
                                                                                                               sacI
                                                                                                               hgiJII
                                                                                                               hgiAI/aspHI
                                                                                                               ecl136II
                                                                                                               bsp1286
                                                                                                               bsiHKAI
                                                                                                               bmyI
                                                                                                          haeIII/palI
                                           ddeI                                                          sau96I aluI
                                          celII/espI                                                     asuI   banII
                       ddeI fnu4HI        bpu1102I                                            hphI      eco0109I/draII
                scfI  mnlI  bbvI       hgaI                                    accI            maeIII   alwNI  ddeI
3401 AGGACAGCAC CTACAGCCTC AGCAGCACCC TGACGCTGAG CAAAGCAGAC TACGAGAAAC ACAAAGTCTA CGCCTGCGAA GTCACCCATC AGGGCCTGAG
     TCCTGTCGTG GATGTCGGAG TCGTCGTGGG ACTGCGACTC GTTTCGTCTG ATGCTCTTTG TGTTTCAGAT GCGGACGCTT CAGTGGGTAG TCCCGGACTC

                                                                   sau96I
                                                                nlaIII
                                                            aciI   haeIII/palI
                                                          fnu4HI   asuI
                                                          bglI styI
                                               aluI      sfiI ncoI
                                              hindIII   eaeI  dsaI                     aluI
                                            tru9I       cfrI   bsaJI                 fnu4HI
            maeIII    aluI                  mseI    taqI haeIII/palI                  bbvI          maeIII
3501 CTCGCCCGTC ACAAAGAGCT TCAACAGGGG AGAGTGTTAA GCTTCGATGG CCGCCATGGC CCAACTTGTT TATTGCAGCT TATAATGGTT ACAAATAAAG
     GAGCGGGCAG TGTTTCTCGA AGTTGTCCCC TCTCACAATT CGAAGCTACC GGCGGTACCG GGTTGAACAA ATAACGTCGA ATATTACCAA TGTTTATTTC

                                                                                                               sau3AI
                                                                                                               mboI/ndeII[dam-]
                                                                                                               dpnI[dam+]
                                                                                                               dpnII[dam-]
                                                                                                              pvuI/bspCI
                                                                                                              mcrI
                                                                                                             taqI[dam-]
                                                                                                            claI/bsp106[dam-]
                                                                                                           sau3AI
                                                                                                           mboI/ndeII[dam-]
                                                                                                           dpnI[dam+]
                                                        rmaI                                               dpnII[dam-]
          sfaNI   apoI                             bsmI maeI                                          nlaIII  alwI[dam-]
3601 CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTCA CTGCATTCTA GTTGTGGTTT GTCCAAACTC ATCAATGTAT CTTATCATGT CTGGATCGAT
     GTTATCGTAG TGTTTAAAGT GTTTATTTCG TAAAAAAAGT GACGTAAGAT CAACACCAAA CAGGTTTGAG TAGTTACATA GAATAGTACA GACCTAGCTA
```


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FIG. 10L

```
                                                                                      rsaI
                        haeIII/palI                                                   csp6I
                       haeI                                                          nlaIV
      tru9I       fnu4HI styI                                                        kpnI
      mseI        bbvI   ncoI                                                        hgiCI
     aseI/asnI/vspI      dsaI                                                        banI                      aluI
    xmnI         hinPI   bsaJI                                                       asp718    mnlI           pvuII
    asp700       hhaI/cfoI nlaIII          mnlI       mnlI                           acc65I  ddeI  aciI       nspBII
3701 CGGGAATTAA TTCGGCGCAG CACCATGGCC TGAAATAACC TCTGAAAGAG GAACTTGGTT AGGTACCTTC TGAGGCGGAA AGAACCAGCT GTGGAATGTG
     GCCCTTAATT AAGCCGCGTC GTGGTACCGG ACTTTATTGG AGACTTTCTC CTTGAACCAA TCCATGGAAG ACTCCGCCTT TCTTGGTCGA CACCTTACAC

                         nlaIV                                                                                    nlaIV
                      scrFI                             sfaNI                  scrFI        scrFI
                      mvaI                                 ppu10I                    mvaI                        mvaI
                      ecoRII                         nsiI/avaIII               ecoRII       ecoRII
                      dsaV                          nlaIII                     dsaV         dsaV
                      bstNI                              sphI                        bstNI                       bstNI
                      apyI[dcm+]                         nspI                        apyI[dcm+]                  apyI[dcm+]
                     bsaJI                               nspHI                      sexAI                       bsaJI
3801 TGTCAGTTAG GGTGTGGAAA GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC CAGGTGTGGA AAGTCCCCAG
     ACAGTCAATC CCACACCTTT CAGGGGTCCG AGGGGTCGTC CGTCTTCATA CGTTTCGTAC GTAGAGTTAA TCAGTCGTTG GTCCACACCT TTCAGGGGTC

                           ppu10I
                           nsiI/avaIII
                          nlaIII
                         sphI
                         nspI sfaNI                                                                               bsrI
                         nspHI                                 aciI          aciI fokI aciI         aciI
3901 GCTCCCCAGC AGGCAGAAGT ATGCAAAGCA TGCATCTCAA TTAGTCAGCA ACCATAGTCC CGCCCCTAAC TCCGCCCATC CCGCCCCTAA CTCCGCCCAG
     CGAGGGGTCG TCCGTCTTCA TACGTTTCGT ACGTAGAGTT AATCAGTCGT TGGTATCAGG GCGGGGATTG AGGCGGGTAG GGCGGGGATT GAGGCGGGTC

                                                              fnu4HI
                                                              bglI
                   nlaIII                                    sfiI
                  styI                                       haeIII/palI
                  ncoI                                     mnlI    mnlI       ddeI
              bslI dsaI                                haeIII/palI bsaJI mnlI   aluI                            mnlI
    aciI      aciI bsaJI                            mnlI bsaJI aciI    haeIII/palI                             mnlI
4001 TTCCGCCCAT TCTCCGCCCC ATGGCTGACT AATTTTTTTT ATTTATGCAG AGGCCGAGGC CGCCTCGGCC TCTGAGCTAT TCCAGAAGTA GTGAGGAGGC
     AAGGCGGGTA AGAGGCGGGG TACCGACTGA TTAAAAAAAA TAAATACGTC TCCGGCTCCG GCGGAGCCGG AGACTCGATA AGGTCTTCAT CACTCCTCCG
```


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FIG. 10M

```
                styI                                                                                    scrFI
                bsaJI                                                                                   mvaI
                blnI                                                                                    ecoRII
             haeIII/palI                  tru9I              haeIII/palI                             dsaV
            stuI rmaI                     mseI              eaeI                                     bstNI
            haeI maeI                    hpaI  aluI         cfrI                    maeIII           apyI[dcm+]        tru9I
           mnlI avrII                aluI hincII/hindII  bsrI                  maeII    bsrI        bsaJI  maeIII     mseI
4101 TTTTTTGGAG GCCTAGGCTT TTGCAAAAAG CTGTTAACAG CTTGGCACTG GCCGTCGTTT TACAACGTCG TGACTGGGAA AACCCTGGCG TTACCCAACT
     AAAAAACCTC CGGATCCGAA AACGTTTTTC GACAATTGTC GAACCGTGAC CGGCAGCAAA ATGTTGCAGC ACTGACCCTT TTGGGACCGC AATGGGTTGA

                                                                 sau3AI
                                                                 mboI/ndeII[dam-]
                                                      sau96I     dpnI[dam+]
                                                      haeIII/palI
                                        aluI          mnlI  aciI  dpnII[dam-]
                fnu4HI                  pvuII         mboII asuI  pvuI/bspCI
                bbvI  fokI              nspBII        earI/ksp632I  mcrI                    bglI
4201 TAATCGCCTT GCAGCACATC CCCCCTTCGC CAGCTGGCGT AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT TGCGTAGCCT GAATGGCGAA
     ATTAGCGGAA CGTCGTGTAG GGGGGAAGCG GTCGACCGCA TTATCGCTTC TCCGGGCGTG GCTAGCGGGA AGGGTTGTCA ACGCATCGGA CTTACCGCTT

      hinPI
      hhaI/cfoI
     nlaIV                                                                                  hinPI
     narI                                                                                   hhaI/cfoI
     kasI                                                                                  thaI
     hinlI/acyI                                                                            fnuDII/mvnI                hinPI
     hgiCI                                                                                 bstUI            hinPI     hhaI/cfoI
     haeII      aciI                                                                       bsh1236I         hhaI/cfoI
     banI   sfaNI                                                                       rsaI      scfI fnu4HI   tru9I
     ahaII/bsaHI                  sfaNI    aciI          aciI  maeII                    csp6I   bslI   aciI     mseI
4301 TGGCGCCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTCACA CCGCATACGT CAAAGCAACC ATAGTACGCG CCCTGTAGCG GCGCATTAAG
     ACCGCGGACT ACGCCATAAA AGAGGAATGC GTAGACACGC CATAAAGTGT GGCGTATGCA GTTTCGTTGG TATCATGCGC GGGACATCGC CGCGTAATTC

                          fnu4HI
        aciI              hinPI
     fnu4HI               hhaI/cfoI                          hinPI
     aciI                thaI                               hhaI/cfoI
    thaI                 fnuDII/mvnI                       haeII
    fnuDII/mvnI          bstUI                          hinPI rmaI
    bstUI                bsh1236I     aciI              hhaI/cfoI    bsrBI
    bsh1236I         maeIII  bbvI maeIII               haeII  maeI   aciI             mboII                  maeII
4401 CGCGGCGGGT GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC CTTCCTTTCT CGCCACGTTC
     GCGCCGCCCA CACCACCAAT GCGCGTCGCA CTGGCGATGT GAACGGTCGC GGGATCGCGG GCGAGGAAAG CGAAAGAAGG GAAGGAAAGA GCGGTGCAAG
```

BNSDOCID <WO 9604391A1 |>

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FIG. 10N

```
                                        nlaIV
      mspI                              hgiJII                                    mnlI
      hpaII                             bsp1286                                nlaIV
     naeI                               bmyI                                   hgiCI
     cfr10I              aluI           banII          nlaIV                   banI   taqI                      hphI
4501 GCCGGCTTTC CCCGTCAAGC TCTAAATCGG GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT TTGGGTGATG
     CGGCCGAAAG GGGCAGTTCG AGATTTAGCC CCCGAGGGAA ATCCCAAGGC TAAATCACGA AATGCCGTGG AGCTGGGGTT TTTTGAACTA AACCCACTAC

        maeII    haeIII/palI
       draIII   sau96I                                    maeII  pleI          tru9I     pleI
       bsaAI    asuI                                     drdI   hinfI maeII   mseI      hinfI          bsrI
4601 GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG TTGGAGTCCA CGTTCTTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC
     CAAGTGCATC ACCCGGTAGC GGGACTATCT GCCAAAAAGC GGGAAACTGC AACCTCAGGT GCAAGAAATT ATCACCTGAG AACAAGGTTT GACCTTGTTG

                                                                                                          thaI
                                                                                                          fnuDII/mvnI
                                                                                                          bstUI
                                                                                        tru9I             bsh1236I
          bslI                                                                          mseI        tru9I       tru9I
         bslI   avaI                                                  haeIII/palI          aluI    mseI   apoI mseI   apoI
4701 ACTCAACCCT ATCTCGGGCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCGGCCTA TTGGTTAAAA AATGAGCTGA TTTAACAAAA ATTTAACGCG
     TGAGTTGGGA TAGAGCCCGA TAAGAAAACT AAATATTCCC TAAAACGGCT AAAGCCGGAT AACCAATTTT TTACTCGACT AAATTGTTTT TAAATTGCGC

                                        hgiAI/aspHI
                                        bsp1286
                      maeII             bsiHKAI                                                                tth111I/aspI
                   tru9I                bmyI    ddeI                    aciI                                   maeIII
       tru9I       mseI                 apaLI/snoI  rsaI                fnu4HI    tru9I                      maeII
       mseI    sspI  psp1406I          alw44I/snoI csp6I          sfaNI       mseI       aciI       bsaAI bsrI
4801 AATTTTAACA AAATATTAAC GTTTACAATT TTATGGTGCA CTCTCAGTAC AATCTGCTCT GATGCCGCAT AGTTAAGCCA ACTCCGCTAT CGCTACGTGA
     TTAAAATTGT TTTATAATTG CAAATGTTAA AATACCACGT GAGAGTCATG TTAGACGAGA CTACGGCGTA TCAATTCGGT TGAGGCGATA GCGATGCACT

                                          hinPI
                                          hhaI/cfoI                mspI
                                        thaI                       hpaII
                hinPI                   fnuDII/mvnI              scrFI                                  bsmAI
              fnu4HI                    bstUI                    nciI     aciI                          esp3I
              bbvI                nspBII  bsh1236I                 dsaV sfaNI                         bslI
       nlaIII  hhaI/cfoI    aciI       aciI  hgaI       drdI      cauII fokI              aluI maeIII
4901 CTGGGTCATG GCTGCGCCCC GACACCCGCC AACACCCGCT GACGCGCCCT GACGGGCTTG TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC
     GACCCAGTAC CGACGCGGGG CTGTGGGCGG TTGTGGGCGA CTGCGCGGGA CTGCCCGAAC AGACGAGGGC CGTAGGCGAA TGTCTGTTCG ACACTGGCAG
```

FIG. 100

```
                                                                thaI
                                                                fnuDII/mvnI
                                                                bstUI
                                                                bsh1236I
      scrFI                                                    hinPI
      nciI                                                     hhaI/cfoI                       mnlI
      mspI       nspI                                         thaI                          haeIII/palI
      hpaII fnu4HI                                            fnuDII/mvnI           mboII    sau96I
      dsaV aluI nspHI                                         bstUI mnlI            bpuAI    asuI
      cauII bbvI nlaIII     mnlI    hphI       hphI           bsh1236I              bbsI    eco0109I/draII
5001 TCCGGGAGCT GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC GAGGCAGTAT TCTTGAAGAC GAAAGGGCCT CGTGATACGC CTATTTTTAT
     AGGCCCTCGA CGTACACAGT CTCCAAAAGT GGCAGTAGTG GCTTTGCGCG CTCCGTCATA AGAACTTCTG CTTTCCCGGA GCACTATGCG GATAAAAATA

                                                                                nlaIV
                                                                              aciI
                                                                             thaI
                                                                             fnuDII/mvnI
                                               maeII                          bstUI
                  nlaIII                      hinlI/acyI                      bsh1236I
        tru9I rcaI                            ahaII/bsaHI                    hinPI
        mseI  bspHI                      ddeI aatII                          hhaI/cfoI
5101 AGGTTAATGT CATGATAATA ATGGTTTCTT AGACGTCAGG TGGCACTTTT CGGGGAAATG TGCGCGGAAC CCCTATTTGT TTATTTTTCT AAATACATTC
     TCCAATTACA GTACTATTAT TACCAAAGAA TCTGCAGTCC ACCGTGAAAA GCCCCTTTAC ACGCGCCTTG GGGATAAACA AATAAAAAGA TTTATGTAAG

                 rcaI
                 bspHI
             bsrBI   bsmAI                                            mboII
             aciI nlaIII                                  sspI        earI/ksp632I
5201 AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT ATTGAAAAAG GAAGAGTATG AGTATTCAAC ATTTCCGTGT CGCCCTTATT
     TTTATACATA GGCGAGTACT CTGTTATTGG GACTATTTAC GAAGTTATTA TAACTTTTTC CTTCTCATAC TCATAAGTTG TAAAGGCACA GCGGGAATAA

                                                                                                    hgiAI/aspHI
                                                                                                    bsp1286
                                                                                          sau3AI    bsiHKAI
                                                                                          mboI/ndeII[dam-]
                                                                                          dpnI[dam+] bmyI
                                                                                          dpnII[dam-]
            fnu4HI                                                                    eco57I        apaLI/snoI
            aciI                            hphI             hphI                 sfaNI mboII[dam-]  alw44I/snoI  maeIII
5301 CCCTTTTTTG CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT GGTGAAAGTA AAAGATGCTG AAGATCAGTT GGGTGCACGA GTGGGTTACA
     GGGAAAAAAC GCCGTAAAAC GGAAGGACAA AAACGAGTGG GTCTTTGCGA CCACTTTCAT TTTCTACGAC TTCTAGTCAA CCCACGTGCT CACCCAATGT
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FIG. 10P

```
                sau3AI                                                                                                  aciI
                mboI/ndeII[dam-]   sau3AI                                                                            thaI
                dpnI[dam+]         mboI/ndeII[dam-]                                                                  fnuDII/mvnI
                dpnII[dam-]        dpnI[dam+]              psp1406I        hgiAI/aspHI                               bstUI
               BstYI/xhoII         dpnII[dam-]             xmnI            bsp1286  tru9I                           bsh1236I
          bsrI          nspBII     alwI[dam-]              asp700          bsiHKAI  mseI                           hinPI
      taqI   alwI[dam-]  aciI   bstYI/xhoII              mboII maeII        bmyI     ahaIII/draI                    hhaI/cfoI
5401  TCGAACTGGA TCTCAACAGC GGTAAGATCC TTGAGAGTTT TCGCCCCGAA GAACGTTTTC CAATGATGAG CACTTTTAAA GTTCTGCTAT GTGGCGCGGT
      AGCTTGACCT AGAGTTGTCG CCATTCTAGG AACTCTCAAA AGCGGGGCTT CTTGCAAAAG GTTACTACTC GTGAAAATTT CAAGACGATA CACCGCGCCA

                        scrFI
                        nciI
                        mspI
                        hpaII
                        dsaV
                        cauII
                   hinlI/acyI                                                                  rsaI
                   hgaI                                 aciI                                   csp6I  bsrI
                   ahaII/bsaHI     bcgI    mcrI fnu4HI          ddeI                          scaI hphI    maeIII      sfaNI
5501  ATTATCCCGT GATGACGCCG GGCAAGAGCA ACTCGGTCGC CGCATACACT ATTCTCAGAA TGACTTGGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT
      TAATAGGGCA CTACTGCGGC CCGTTCTCGT TGAGCCAGCG GCGTATGTGA TAAGAGTCTT ACTGAACCAA CTCATGAGTG GTCAGTGTCT TTTCGTAGAA

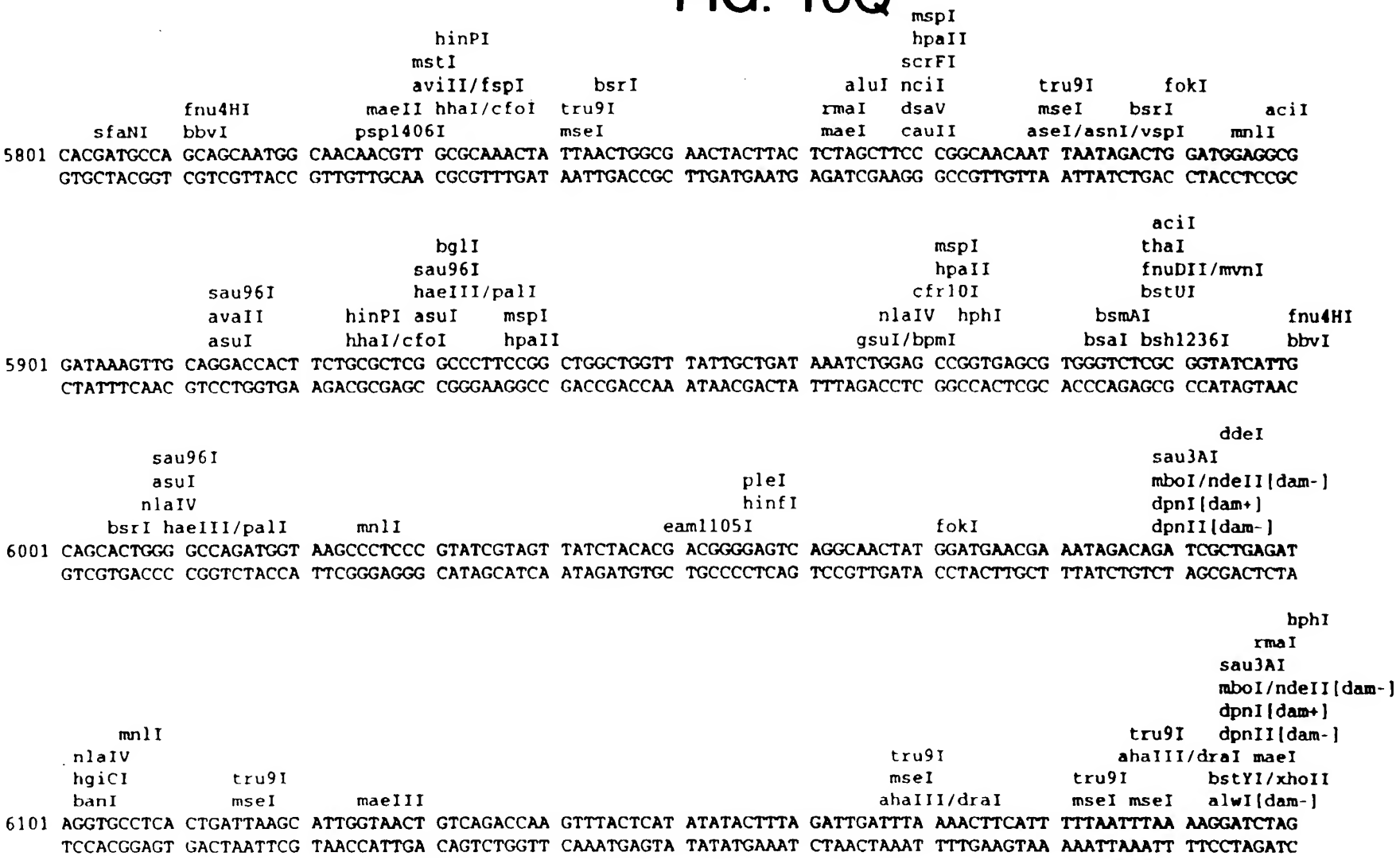
                                                                                                                  sau96I
                                                                                                                  avaII
                                                                                                        sau3AI    asuI
                                                                                haeIII/palI                       mboI/ndeII[dam-]
                                                                               eaeI                               dpnI[dam+]
                                                                               cfrI                               dpnII[dam-]
                                        fnu4HI                                 fnu4HI                             pvuI/bspCI
        fokI  nlaIII                    bbvI          nlaIII                   aciI                               mcrI  mnlI
5601  ACGGATGGCA TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG TGATAACACT GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG
      TGCCTACCGT ACTGTCATTC TCTTAATACG TCACGACGGT ATTGGTACTC ACTATTGTGA CGCCGGTTGA ATGAAGACTG TTGCTAGCCT CCTGGCTTCC

                                      nlaIII
                                   sau3AI maeIII                        mspI
                                   mboI/ndeII[dam-]     sau3AI   nlaIV
                                   dpnI[dam+]           mboI/ndeII[dam-]
                                  alwI[dam-]            dpnI[dam+]   hpaII
      aluI  aciI                nlaIII dpnII[dam-]      dpnII[dam-] bsaWI aluI                                      maeIII
5701  AGCTAACCGC TTTTTTGCAC AACATGGGGG ATCATGTAAC TCGCCTTGAT CGTTGGGAAC CGGAGCTGAA TGAAGCCATA CCAAACGACG AGCGTGACAC
      TCGATTGGCG AAAAAACGTG TTGTACCCCC TAGTACATTG AGCGGAACTA GCAACCCTTG GCCTCGACTT ACTTCGGTAT GGTTTGCTGC TCGCACTGTG
```

SUBSTITUTE SHEET (RULE 26)

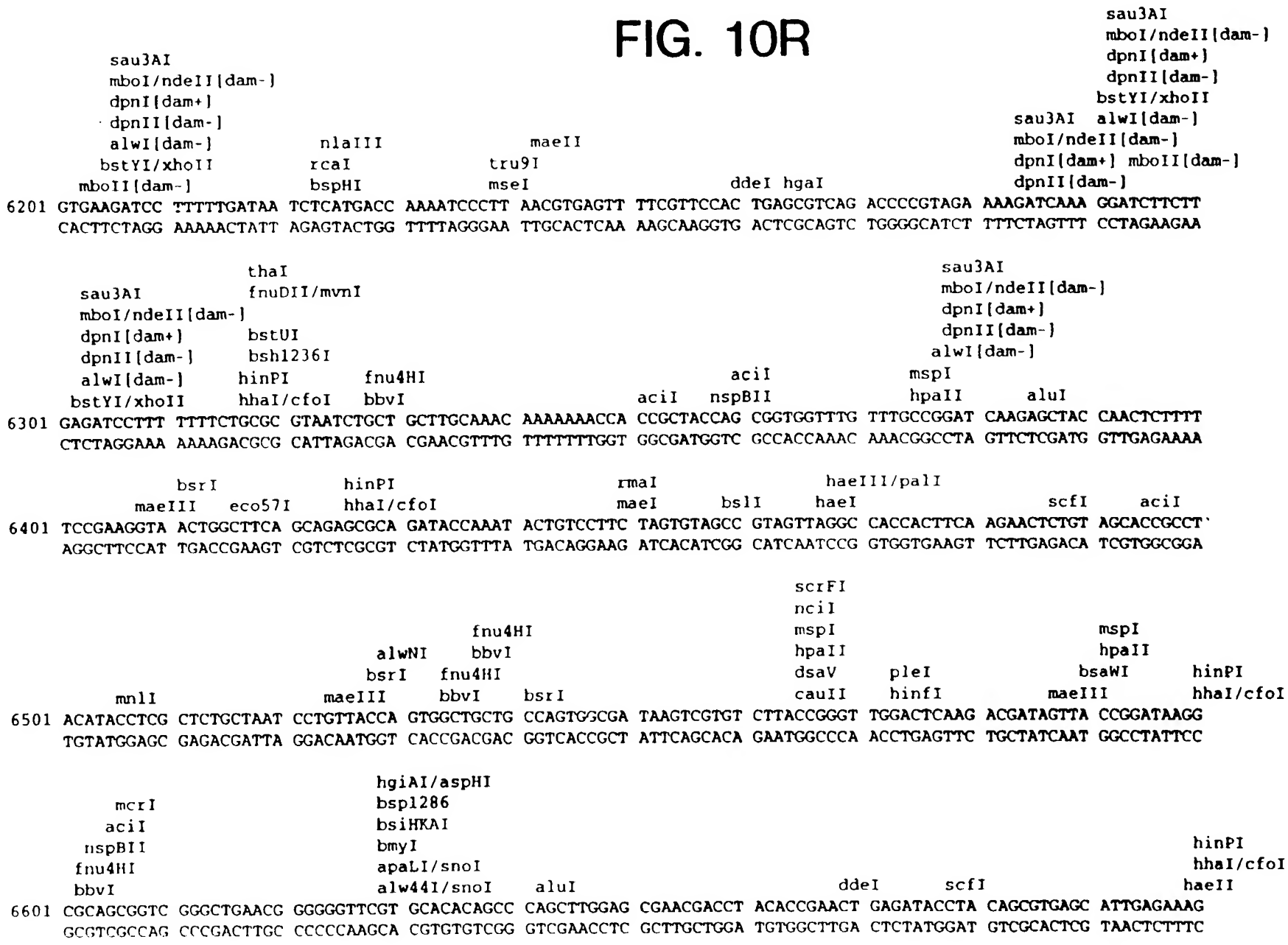
BNSDOCID <WO 9604391A1_I_>

FIG. 10Q



BNSDOCID <WO 9604391A1_I_>

FIG. 10R



SUBSTITUTE SHEET (RULE 26)

FIG. 10S

```
                                                                                               scrFI
                                                                                               mvaI          scrFI
                                                                                               ecoRII  mvaI
                                             mspI                                              dsaV    ecoRII
                                             hpaII                                             bstNI         dsaV
                                             bslI    fnu4HI                  hinPI  mnlI       bsaJI         bstNI
                                aciI        bsaWI    aciI                    hhaI/cfoI   aluI  apyI[dcm+]    apyI[dcm+]
6701 CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG
     GCGGTGCGAA GGGCTTCCCT CTTTCCGCCT GTCCATAGGC CATTCGCCGT CCCAGCCTTG TCCTCTCGCG TGCTCCCTCG AAGGTCCCCC TTTGCGGACC

                                                                                                                 fnu4HI
                                                                                                                 aciI
                                                                                                                thaI
                                                                                                                fnuDII/mvnI
                                                     taqI                               nlaIV                   bstUI
                                    mnlI  drdI    hgaI           sfaNI            aciI                          bsh1236I
6801 TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAAC GCCAGCAACG
     ATAGAAATAT CAGGACAGCC CAAAGCGGTG GAGACTGAAC TCGCAGCTAA AAACACTACG AGCAGTCCCC CCGCCTCGGA TACCTTTTTG CGGTCGTTGC

                         haeIII/palI
                      scrFI
                      mvaI
                      ecoRII
                      dsaV                    nlaIII
                      bstNI bslI                   nspI
        bslI          apyI[dcm+]      haeIII/palI  nspHI                       tfiI
      haeIII/palI nlaIV haeI        haeI           aflIII                      hinfI                    aciI
6901 CGGCCTTTTT ACGGTTCCTG GCCTTTTGCT GGCCTTTTGC TCACATGTTC TTTCCTGCGT TATCCCCTGA TTCTGTGGAT AACCGTATTA CCGCCTTTGA
     GCCGGAAAAA TGCCAAGGAC CGGAAAACGA CCGGAAAACG AGTGTACAAG AAAGGACGCA ATAGGGGACT AAGACACCTA TTGGCATAAT GGCGGAAACT
```


BNSDOCID <WO___9604391A1_I_>

FIG. 10T

```
                                                                                                                 chaI
                                                                                                                 fnuDII/mvnI
                                                                                                                 bstUI
                                                                                                                 bsh1236I
                                                                                                                hinPI
                                                                                                                hhaI/cfoI
                                                                                                               thaI
                                                                                               hinPI           fnuDII/mvnI
                        fnu4HI             fnu4HI                                         sapI hhaI/cfoI       bstUI
                        bbvI               bbvI   pleI                                    mboII                bsh1236I
                bsrBI  aciI              hinPI    hinfI                                   earI/ksp632I   mnlI  bslI
      aluI      aciI  fnu4HI      mcrI   hhaI/cfoI                 mnlI       aciI   haeII            aciI   aciI
7001 GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC GACCGAGCGC AGCGAGTCAG TGAGCGAGGA AGCGGAAGAG CGCCCAATAC GCAAACCGCC TCTCCCCGCG
     CACTCGACTA TGGCGAGCGG CGTCGGCTTG CTGGCTCGCG TCGCTCAGTC ACTCGCTCCT TCGCCTTCTC GCGGGTTATG CGTTTGGCGG AGAGGGGCGC

                    tru9I  aluI
       haeIII/palI         pvuII                                                    tru9I                 nlaIV
      eaeI tfiI     mseI   nspBII                                      hinPI        mseI      maeIII      hgiCI
      cfrI hinfI aseI/asnI/vspI               bsrI      aciI           hhaI/cfoI  aseI/asnI/vspI mnlI     banI
7101 CGTTGGCCGA TTCATTAATC CAGCTGGCAC GACAGGTTTC CCGACTGGAA AGCGGGCAGT GAGCGCAACG CAATTAATGT GAGTTACCTC ACTCATTAGG
     GCAACCGGCT AAGTAATTAG GTCGACCGTG CTGTCCAAAG GGCTGACCTT TCGCCCGTCA CTCGCGTTGC GTTAATTACA CTCAATGGAG TGAGTAATCC

       scrFI
       mvaI
       ecoRII
       dsaV
       bstNI
       apyI[dcm+]             mspI                          aciI                                        xmnI
      bsaJI                   hpaII                        bsrBI                        aluI     nlaIII  asp700
7201 CACCCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATGTT GTGTGGAATT GTGAGCGGAT AACAATTTCA CACAGGAAAC AGCTATGACC ATGATTACGA
     GTGGGGTCCG AAATGTGAAA TACGAAGGCC GAGCATACAA CACACCTTAA CACTCGCCTA TTGTTAAAGT GTGTCCTTTG TCGATACTGG TACTAATGCT

       tru9I
       mseI
     aseI/asnI/vspI
7301 ATTAA
     TAATT

>length: 7305
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BNSDOCID <WO___9604391A1_I_>

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/09576

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/64 C12N15/67 C12N15/85 C12N9/72 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DNA CLONING, VOLUME III, EDITED BY D.M. GLOVER, 1987 IRL PRESS, OXFORD, GB;, pages 189-212, A.M.C. BROWN AND M.R.D. SCOTT 'Retroviral vectors'	1-3,7,8
Y	see page 192, line 7 - page 196, line 5; figures 2,3	5,6, 9-12, 16-21
	--- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search
23 November 1995

Date of mailing of the international search report
08.12.95

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Fax (+ 31-70) 340-3016

Authorized officer
Hornig, H

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CELL, vol. 37, no. 3, July 1984 CELL PRESS,CAMBRIDGE,MA,US;, pages 1053-1062, C.L. CEPKO ET AL. 'Construction and applications of a highly transmissible murine retrovirus shuttle vector' cited in the application	1-3,7,8
Y	pZIP-Neo SV(B)1 see figure 1	5,6, 9-12, 16-21

Y	MOL. CELL. BIOL., vol. 5, no. 3, March 1985 ASM WASHINGTON, DC,US, pages 431-437, A.D. MILLER ET AL. 'Generation of helper-free amphotrophic retroviruses that tranduce a dominant-acting, methotrexate-resistant dihydrofolate reductase gene' see page 432, right column, line 5 - page 436, right column, line 7; figure 1	5,6, 9-12, 16-21

Y	WO,A,94 05784 (US) 17 March 1994 see the whole document	5,6, 9-12, 16-21

Y	EP,A,0 215 548 (ZYMOGENETICS INC ;UNIV WASHINGTON (US)) 25 March 1987 see the whole document	5,6, 9-12, 16-21

A	WO,A,92 17566 (GENENTECH INC) 15 October 1992 cited in the application see the whole document	1-21

A	WO,A,90 12025 (UNIV LELAND STANFORD JUNIOR) 18 October 1990 cited in the application see the whole document	1-21

A	EP,A,0 260 148 (GENENTECH INC) 16 March 1988 see the whole document	1-21

A	EP,A,0 160 457 (GENENTECH INC) 6 November 1985 cited in the application see the whole document	1-21

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BNSDOCID <WO___9604391A1_I_>

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/US 95/09576

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROC. NATL.ACAD SCI., vol. 86, February 1989 NATL. ACAD SCI.,WASHINGTON,DC,US;, pages 1041-1045, M. VIVAUD ET AL. 'A 5' splice-region G-C mutation in exon 1 of the human beta-globin gene inhibits pre-mRNA splicing: A mechanism for beta+-thalassemia' see the whole document	1-4

2

Form PCT ISA 210 (continuation of second sheet) (July 1992)

BNSDOCID: <WO___9604391A1_I_>

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/US 95/09576

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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		DE-T-	3688900	09-12-93
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		US-A-	4959318	25-09-90
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		GB-A,B	2197321	18-05-88
		US-A-	5024939	18-06-91
		JP-A-	63152986	25-06-88
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		AU-B-	4134585	24-10-85
		AU-B-	5295890	30-08-90
		EP-A-	0385558	05-09-90
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		JP-A-	60243023	03-12-85
		JP-A-	6040942	15-02-94
		NO-B-	174934	25-04-94
		SG-A-	3994	10-06-94
		US-A-	4965199	23-10-90

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